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WELCOME MESSAGE

Welcome to Aquaculture Europe 2022 on the Italian Adriatic coast in Rimini.

The theme of the conference "Innovative Solutions in a Changing World" reflects the need for addressing the many challenges facing the sector in the coming decades. Most in land, coastal and marine water bodies will undoubtedly be impacted directly or indirectly by climate change and urbanisation, from sea acidification and warming, sea-level rise, coastal erosion, flooding, eutrophication and pollution. These will represent important sustainability challenges for current and future European aquaculture. AE2022 will provide a great opportunity for discussing new and innovative ideas to address these challenges but also identify strategies for implementing and up scaling already proven concepts and solutions.

Since moving recently from academia to industry, I can testify on the importance of capturing and translating new research into industry protocols and solutions for the benefit of the sector. What makes EAS annual events unique is bringing together scientists, industry leaders and entrepreneurs, governmental bodies and regulators from all over Europe and sharing the same passion for aquaculture. AE2022 will include a wide range of scientific sessions (32 over 3 days) and a trade show with close to 180 booths. In addition, several workshops and special events will take place; including the AE2022 Industry Forum and the AE2022 Innovation Forum - organised by The European Aquaculture Technology and Innovation Platform, the European Commission and EAS.

This year we are expecting more than 2000 attendees with more than 600 scientific abstracts received and these have been reviewed by the session chairs and integrated into an impressive programme by Maria Letizia Fioravanti and Daniel Żarski as 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 AE2022 possible as for my colleagues on the Board of the EAS with several newly appointed directors. A big thanks also to our Gold Sponsors Biomar, Silver Sponsors U.S. Soybean Export Council, Session Sponsor Lallemand and Conference Support from the Italian National and Regional Governments.

This is the end of my two-year term as President. It has been a challenging period for all, but I am delighted to see the great resilience of EAS thanks to our collective efforts and welcome to our new president, Bente Torstensen. I hope you enjoy the event, the people and the science.

Herve Migaud
EAS President 2020-2022

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ABSTRACTS

THE EFFECT OF FOAM FRACTIONATION AND OZONATION TO WATER QUALITY AND MICROBIAL COMMUNITIES IN RAS

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Introduction

Microbes inhabit all the compartments of recirculating aquaculture systems (RAS), being suspended in water phase and forming biofilms on the surfaces. The majority of these microbes is considered to be harmless for the fish, and some of them are even beneficial, having an important role in the nitrifying biofilters. However, uncontrolled growth of microbial biomass can promote the abundance of harmful microbes, and increases the need for aeration and degassing. To maintain sufficient water quality and to hinder microbial blooms, water treatment methods to remove microbes and other organic matter are currently searched and developed for RAS. Two potential methods are foam fractionation and ozonation, the first removing organic matter, while the latter destroys and inactivates microbes directly. In this study, we examined the effect of foam fractionation and/or ozonation on water quality and on microbial communities in water and biofilter biofilms in replicated freshwater RAS with rainbow trout.

Materials and methods

The experiment was conducted in 12 replicated, 0.8 m^3 pilot-scale freshwater RAS stocked with 8.05 ± 0.03 kg juvenile rainbow trout (*Oncorhynchus mykiss*) and with final feed loading of 1.66 kg feed m^{-3} make-up water. Four treatments were applied in triplicate: 1) three control RAS, 2) three RAS with foam fractionator (ff), 3) three RAS with ozone (oz), 4) and three RAS with ozone and foam fractionator (oz + ff). The trial lasted eight weeks. Water quality characteristics, and microbial activity and abundance were measured every week. The composition of microbial communities in water was analysed at beginning of the experiment (week 0) and at weeks 1, 3, and 7 and the composition of communities in biofilter biofilms at week 7 using next generation sequencing with IonTorrent PGM targeted on 16S rRNA gene V4 region. The detailed description of the experimental setup and sample analysis is given in de Jesus Gregersen et al. (2021) and Aalto et al. (2022)”ISSN”:”00448486”,”abstract”:”Efficient water treatment is required to maintain high water quality and control microbial growth in recirculating aquaculture systems (RAS.

Results and discussion

Both ozonation and foam fractionation improved the system water quality by decreasing the biological and chemical oxygen demand and the amount of microparticles in water (Table 1), and this effect was pronounced when these two water treatments were combined. The same trend was observed with microbial activity, which decreased by 10-fold when applying both ozonation and foam fractionation as compared to the control systems. However, foam fractionation affected neither the microbial community composition (Fig. 1A) nor the abundance of microbes (data not shown), suggesting that the lower microbial activity observed under foam fractionation was due to the lower amount of organic matter being available for heterotrophic microbes. In constrast, ozone posed a strong selection pressure by attacking the microbes directly, having a significant effect on the microbial community composition already one week after application (Fig. 1). Moreover, the microbial abundance was significant lower in ozonated units (oz, oz + ff) than in foam fractionation or control units (data not shown). While the biofilter biofilm communities were more resistant to the water treatment than the communities suspended in the system water, we found comammox *Nitrospira*, carrying out the complete nitrification (Daims et al., 2015), to be more abundant in the foam fractionation unit biofilters. This together with the significantly lower nitrite concentration found in the foam fractionation treatment (Table 1) suggests that by removing organic matter, foam fractionation promotes more stable nitrification in the biofilters by decreasing the activity of heterotrophs, which allows a higher abundance of nitrite oxidizers but also a shift in the nitrifying community from canonical two-step process into the complete nitrification. Altogether, the results indicate that although these two water treatment methods have similar outcomes on physico-chemical water quality and microbial activity, their underlying mechanisms are different, potentially leading to different outcomes under the long-term application.

(Continued on next page)

Table 1. Water quality characteristics in four triplicate treatments. Values are given as mean±SD over experimental weeks 6-8 (n = 9/treatment).

	Control	Foam fractionator	Ozone	Ozone + Foam fractionator
TAN (µg NH_4-N/L)[3]	74.7 ± 31.8	83.8 ± 19	88.5 ± 33.5	82.9 ± 12.3
Nitrite (µg NO_2-N/L)	119 ± 26.0^{a}	77.5 ± 21.8^{bc}	104 ± 16.5^{ab}	70.5 ± 8.4^{c}
Nitrate (mg NO_3-N/L)	57.5 ± 2.71	56.7 ± 2.85	57.4 ± 2.64	56.6 ± 2.81
BOD5 (mg O_2/L)[2]	6.09 ± 1.10^{a}	2.99 ± 0.94^{b}	3.45 ± 0.59^{b}	1.53 ± 0.26^{c}
COD (mg O_2/L	37.6 ± 6.22^{a}	22.8 ± 2.86^{b}	25.2 ± 3.03^{b}	16.1 ± 1.50^{c}
Particle abundance (10^6/mL)	2.43 ± 1.46^{a}	1.01 ± 0.63^{a}	0.42 ± 0.24^{b}	0.27 ± 0.15^{b}
Microbial activity k (h^{-1})[1]	0.84 ± 0.26^{a}	0.33 ± 0.18^{b}	0.44 ± 0.29^{ab}	0.08 ± 0.03^{c}

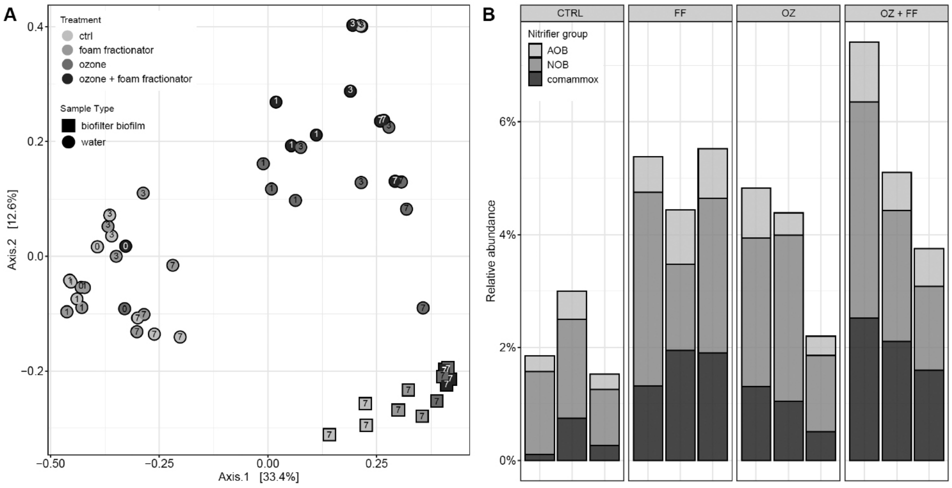

Figure 1. A) PCoA of microbial communities based on Bray-Curtis similarities and B) relative abundance of nitrifying microbes in the biofilter biofilm samples between four different treatments.

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DIFFERENTIAL EXPRESSION ANALYSIS OF SEX GENES DURING PINE POLLEN-INDUCED MASCULINIZATION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

Due to the risks associated with using synthetic chemicals in producing all-male tilapia seed, plant-based products are presently considered as safe nature-based alternatives. In the present study, pine pollen (PP) was utilized to sex inverse Nile tilapia (*Oreochromis niloticus*), since it contains phytoandrogens that have potential to alter the process of sex differentiation in fish. However, there is no available data describing the molecular mechanism involved in PP-induced sex inversion of Nile tilapia. To understand the role of PP, the study examined its effect on the expression of sex-related genes: cyp19a1a, foxl2, dmrt1 and amh, in all-female (XX) individuals, which were fed a basal diet supplemented with 1.28 g PP kg^{-1}. This was compared to the expression patterns obtained from fish fed: 1) only a basal diet (CT treatment) and 2) a basal diet supplemented with 0.06 g 17α-methyltestosterone (MT) kg^{-1}. Generally, the expression levels of all genes were altered. In the fish treated with PP and MT, significant up-regulation of dmrt1 and amh was observed, but were repressed in the CT treatment ($P < 0.001$). At the same time, cyp19a1a and foxl2 were markedly down-regulated in the PP and MT treatments, and up-regulated in the CT group ($P < 0.001$). While dmrt1 and amh were activated in the PP-treated fish, higher expression was observed in the MT group. This expression pattern was consistent with masculinization levels in the PP and MT treatments. A significantly higher proportion of male individuals (97.8 ± 1.1 %) was obtained in the MT treatment compared to 77.8 ± 2.9 % in the PP group ($P = 0.003$). However, all the female fish in the CT group remained un-masculinized. Therefore, cyp19a1a and foxl2 were female-biased while dmrt1 and amh were mainly associated with maleness, confirming their role in female and male sex development respectively. These findings suggest that PP induces sex masculinization of Nile tilapia by activating the expression of both dmrt1 and amh accompanied by attenuation of foxl2 and cyp19a1a.

EFFECT OF A MIXTURE OF *Yucca schidigera* EXTRACT AND *Bacillus subtilis* ON THE AMMONIA REDUCTION IN EFFLUENT AND ANTIOXIDANT ACTIVITY OF NILE TILAPIA (*Oreochromis niloticus*)

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Tilapia (*Oreochromis niloticus*) farming effluents contain high level of ammonia, which poses a significant threat to fish and the surrounding environment. *Yucca schidigera* extract (YE) has been found to reduce ammonia pollution resulting from fish farming. On the other hand, dietary supplementation of *Bacillus subtilis* was found to improve anti-oxidation activity, and water ammonia level for fish. Therefore, the aim of the present study was to assess an inclusion of *Yucca schidigera* extract and *Bacillus subtilis* separately or in combination on anti-oxidation activity and ammonia reduction in the effluent of Nile tilapia fingerlings.

Fish were divided into 4 groups and fed for 60 days with four experimental diets which supplemented with 0.2 gkg^{-1}diet of YE and 2 g kg^{-1}diet of *Bacillus subtilis* (B) separately or in combination (YEB) and the control diet was left without addition (CON).

Results revealed the positive role of YE and B on total ammonia nitrogen (TAN) reduction (Table 1); however combination of both YE and B had less efficiency. The addition of YE or B in tilapia diets reduced TAN removing by 50% compared with the control. Superoxide dismutase (SOD) showed the best activity in YEB treatment followed by YE and B treatments while the lowest activity was recorded in the control group. Fig. 1 illustrates the effect of YE and B on anti-oxidative activity of Nile tilapia.

The highest activity of Malondialdehyde (MDA) was reported in the control group while the lowest value was found in YEB group followed by YE and then B group. Glutathione peroxidase (GPx) and total antioxidant capacity (TAC) showed the highest activity in YEB followed by B treatment, while the lowest activity was recorded in YE and CON. It could be concluded that Yucca extract and bacillus can efficiently reduce ammonia in Nile tilapia effluent and boost their antioxidative activity.

Table 1. Effects of experimental diets on antioxidant activity of Nile tilapia

Treatment	SOD	MDA	GPx	TAC
CON	16.63±0.55^{c}	4.07 ±0.15^{a}	0.23±0.03^{c}	0.25±0.04^{d}
YE	24.17 ±0.25^{b}	3.27 ±0.15^{b}	0.33±0.02^{c}	0.60±0.10^{c}
B	25.30 ±0.66^{b}	2.400 ±0.10^{c}	0.63±0.06^{b}	1.33±0.15^{b}
YEB	31.63±1.19^{a}	1.03±0.15^{d}	1.77±0.15^{a}	2.27±0.14^{a}

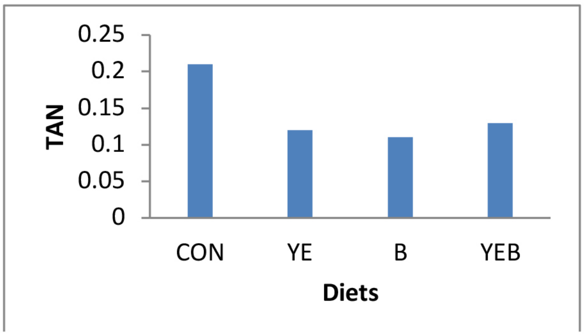

Fig. 1. Effects of experimental diets on TAN of effluent of Nile tilapia

ARE FUGITIVE FISH ESCAPING FROM MANAGEMENT? A MULTI-STAKEHOLDER APPROACH TO TACKLE ESCAPE EVENTS

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Background

During 2021 the GLORiA project (Global Change Resilience in Aquaculture) identified socioeconomic impacts and interactions of escapees with the surrounding environment and studied de frecuency, magnitude and causes to anticipate future scenarios derived from global change. GLORiA increased the knowledge of the origin of escaped fish at fish markets, wholesale and retailers established and made it possible to establish the scientific criteria to be taken into account when establishing a contingency plan.

GLORiA^2 is an extension of the research carried out in GLORiA. During the implementation of this project, a series of needs were raised by the stakeholders (aquaculture companies, fishing sector, other scientific bodies, regional and national administrations) which require to continuously develop actions to improve our knowledge about the impacts of escapes both in the environment and in the value chain; from the moment the cages suffer a technical failure to that moment when the fish is purchased by the final consumer (Fig.1).

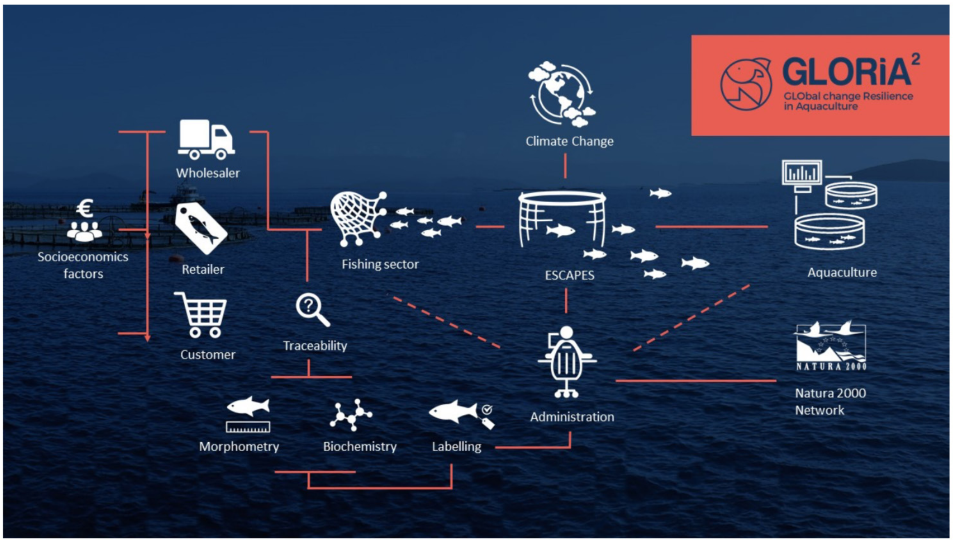

Figure 1. Conceptual framework from GLORiA 2 project

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GLORiA² project goals

- Encourage participatory management through the creation of working groups at regional and national levels.
- Use state-of-the-art computer vision and analysis technologies to improve the identification of escaped fish at the fish market and therefore their traceability.
- Improve, through morphometric and physiological biomarkers, the traceability of escaped fish entering value chains in order to ensure food safety for consumers.
- Analyse the level of fraud in products shared by aquaculture and extractive fishing, proposing measures related to labelling in order to allow correct identification.
- Predicting future incidence of fish escape events and their interactions with marine coastal ecosystems.
- Analyse social preferences about escaped fish management measures and their implications for fresh fish market prices, improving food security and environmental sustainability.

Acknowledgements

GLORiA 2 is developed with the collaboration of the Biodiversity Foundation (Spanish Ministry for Ecological Transition and the Demographic Challenge), through the Pleamar Program, co-financed by the European Maritime and Fisheries Fund (EMFF).

NORMALIZING DATA MODELS FOR INTEROPERABILITY IN AQUACULTURE

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Introduction

Smart feeding systems are emerging in aquaculture. They are expected to bring improvement and optimization of feeding practices by digitizing real-time biological and environmental information, integrated with core biological knowledge, new technologies and machine learning to maximize profit while minimizing environmental footprint and maintaining fish welfare.

This digitization requires the automation of data collection, exchange, and decision support. In such a process, sensor data are collected, combined with other important data such as fish behaviour, feeding patterns, weather conditions, and finally processed by Artificial Intelligence (AI) algorithms to provide recommendations and management decisions, such as feeding management strategies and optimal harvest times.

The issue if that the offers are multiplying to provide vertically integrated solutions with limited capabilities for the end user to share data from one application to one another. This creates several drawbacks including the risk of vendor lock-in, the limited innovation capabilities or having a sub-optimal system not allowing data reuse.

This could be achieved by collecting and validating data from heterogeneous sources and organise it in an interoperable way so it can be used over several applications spanning over different domains. In this paper and following our previous work in [1], we will introduce our work toward the normalization of Aquaculture Data model for smart fish feeding support using standardised cross-domain specifications.

Data Platform

For managing data in a fish farming system, the proposed core data platform is based on NGSI-LD[1]. The NGSI-LD specification is produced by ETSI and made open-source within the FIWARE ecosystem[2]. From a technical perspective, FIWARE brings a curated framework of open-source software components which can be assembled and combined with other third-party platform components to build platforms easing the development of smart solutions and smart organizations.

In a nutshell, the NGSI-LD specification is based on data model and API. The NGSI-LD data model is an entity-based data model. An NGSI-LD Entity is an informational representation that is supposed to exist physically or conceptually. Relationships in NGSI-LD capture possible links between a subject which maybe an entity, a property, or another relationship on the one hand, and an object, which is an entity, on the other hand. NGSI-LD Property is a description instance, which associates a main characteristic to either an Entity, a Relationship, or another Property. Based on its cross-domain ontology, NGSI-LD covers several generic domains such as Mobility, Location, Temporal, System Structure and Behavioural Systems. The NGSI-LD API relies on this data model. It provides a set of operations on entities covering entity creation, update, deletion, retrieval, and subscription. The API also proposes operations that include temporal operations.

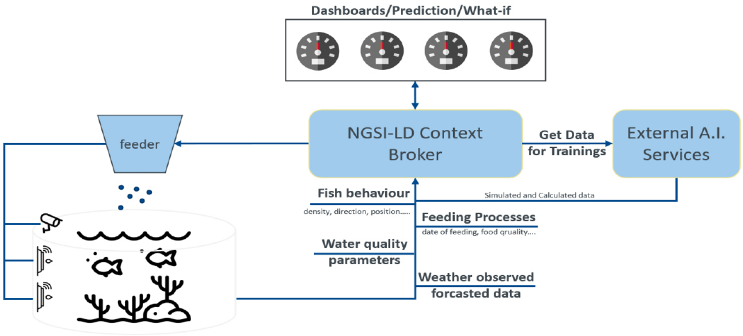

Figure 1: Overall schematic of Data Management in a fish farming system.

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A simplified overall schematic of Data management in a fish farming system is depicted in Figure 1. The main NGSI-LD entity in this fish farming system is the Fish Containment: Fish Cage, or Tank, are the equivalent concept for it. Data may be devised in different categories: Fish Behaviour, Feeding Processes, Water Quality and Weather. Weather Parameters (temperature, wind, cloud, waves height, etc.) will be added as a property to Weather Observed Entity. Water Parameters (pH, redox, dissolved Oxygen…) are added as properties to the Fish Containment entity.

We analysed a number of existing data models and ontologies including Agrovoc[3], WORMS[4], dbpedia; FOODI[5], Aquacloud[6], SmartdataModels[7] to identify terms relevant for the aquaculture applications, list equivalence among the models and select a definition leading to the least unambiguous description of the term. This term is then mapped over the NGSI-LD interface so data exchanges can occur across the systems. The table below illustrates the discussion for one NGSI-LD entity and one NGSI-LD property. The complete property graph will be described in detail during the presentation.

Examples of mapped concepts

Examples of mapped concepts

attributes	NGSI-LD type	Links In External Knowledges	Equivalent Terms
feedingOperation	Entity	http://aims.fao.org/aos/agrovoc/c_2922	Feeding procedure,
Feeder	Entity-	http://aims.fao.org/aos/agrovoc/c_29bc6f3f	Feeding equipment, Fish feeder, Feed dispenser

Conclusion and Discussion

The NGSI-LD fish farming system is published in the smart data models repository[8]. In this data model we have tried to normalize data from different sources and types in one common NGSI-LD model. We have also tried to link and map almost data model attributes to external knowledge and known thesaurus for interoperability issue.

Reference

[1] Abid, A., Dupont, C., Le Gall, F., Third, A., & Kane, F. (2019, June). Modelling Data for A Sustainable Aquaculture. In 2019 Global IoT Summit (GIoTS) (pp. 1-6). IEEE.

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https://www.dbpedia.org/
https://aquacloud.ai/sensor-data/smartdatamodels.org/
https://smartdatamodels.org/
https://github.com/smart-data-models/dataModel.Aquaculture

EFFECT OF SHADED AND NON-SHADED CULTURE CONDITIONS ON GROWTH PERFORMANCE AND FEED CONVERSION RATIO OF JUVENILE AND PRE-ADULT GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

The effect of light intensity on the growth performance, feed utilization and stress response have been studied in different fish species, including gilthead seabream (Sparus aurata), and European seabass (Dicentrachus labrax) (Tandler and Mason 1983; Cuvier-Peres et al., 2001). High light intensity can contribute to fish stress, which increase the energy expenditure and reduce growth rates and feeding efficiency (Karakatsouli & Chiras 2005). There is a paucity of research in evaluating the growth performance and feed utilization of gilthead seabream fed different diets under shaded and non-shaded conditions. Therefore, in this study we compared the effects of shaded and non-shaded culture condition on the growth performance of gilthead seabream juvenile fed with commercial diet and in-house formulated improved diets.

Materials and Methods

Four test diets, improved diet (ID) and commercial diet (CD) for juvenile gilthead seabream, similarly, improved diet (ID) and commercial diet (CD) for pre-adult gilthead seabream were used in this experiment. In experiment 1, eighteen juvenile gilthead seabream (average weight of 55.23 ± 1.08 g and total length of 14.07 ± 0.16 cm) were stocked in triplicates in each tank at density 4 kg m-3. In experiment 2, forty three pre-adult gilthead seabream (average weight of 234.40 ± 2.57 g and total length of 23.35 ± 0.07 cm). In each experiment, the four different treatments were carried out for juvenile and pre-adult stage of gilthead seabream 1) shaded condition, fish fed improved diet (SID); 2) Non-shaded condition, fish fed improved diet (NID) 3) shaded condition, fish fed commercial diet (SCD) 4) non-shaded condition, fish fed commercial diet (NCD). For the shaded treatment, the tanks were covered by 90% green shade net to cut off the natural light intensity. For the non-shaded treatment, the tanks were exposed to the natural light. The light intensity in all the treatments (shaded and non-shaded) were measured by Lux meter and the unit was expressed in lux (lx). Fish were hand-fed at apparent satiation daily twice a day (9:00 - 15:00), except the days of sampling when the fish were kept under starvation. Pellets were released slowly to allow fish to easily chase the food and to evenly eat. Feed intake was recorded daily and uneaten pellets remained in the tanks were removed and air-dried, and pellets were weighed to calculate daily fish feed intake. The experiment lasted for 74 days. The proximate composition of the commercial diets and fish samples were estimated following the standard methods (AOAC). All data was analyzed using ANOVA followed by Fisher Least Significant Difference (LSD) test.

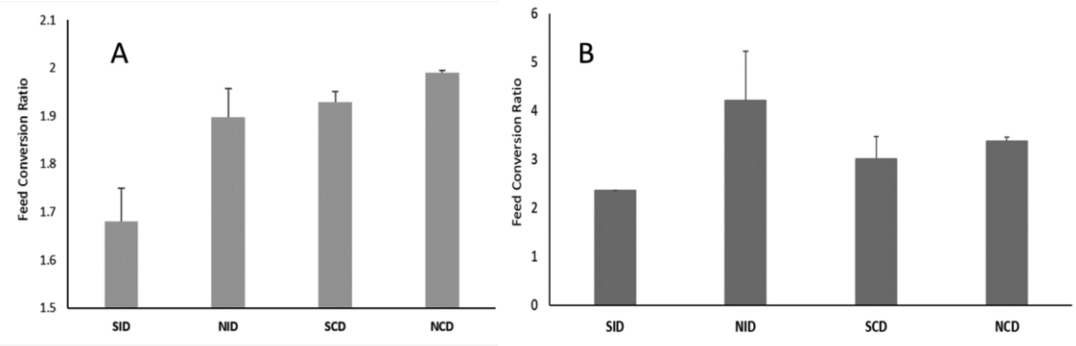

Figure 1: Feed conversion ratio of A: juvenile gilthead seabream B: pre-adult gilthead seabream reared under different conditions like shaded with improved diet (SID), non-shaded with improved diet (NID), shaded with commercial diet (SCD) and non-shaded with commercial diet (NCD).

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Results

The survival of the juvenile gilthead seabream fed improved diet under shaded condition was high (100%). The growth of the juvenile gilthead seabream has increased 2.2 - 2.4 times from its initial size after 74 days of culture. The final weight gain of juvenile fish showed significant difference ($P < 0.05$) among the different treatments. The FCR of juvenile gilthead seabream was significantly lower ($P = 0.002$) under the shaded condition where fish fed with improved diet. The SGR of the shaded treatment fed with improved diet showed significantly higher when compared to the non-shaded treatments. In pre-adult gilthead seabream, the feed conversion ratio was lower (2.36) in shaded treatment fed with improved diet (SID) as compared to other treatments. One way ANOVA analysis revealed that survival, final body weight gain and SGR are significantly higher in shaded condition where fish fed improved diet.

Discussion

In this present study, our results showed that juvenile and pre-adult gilthead seabream attained high survival rate (100%) when fed with improved diets under shaded conditions. However, the survival rate of juvenile gilthead seabream is not statistically significant between shaded and non-shaded conditions. Similar results were observed on juvenile catfish Clarias gariepinus and Epinephelus coioides, where the light intensity did not influence the survival rate (Tao et al., 2013). Alvarez-Verde et al., (2015) reported that Brazilian flounder *Paralichthys orbignyanus* prefer low light intensity for better growth. Similarly, in the present study, the final weight gain of fish, feed conversion ratio and specific growth rate of juvenile and pre-adult gilthead seabream were influenced significantly in shaded condition where fish fed with improved diet.

Conclusions

In conclusion, survival and growth performance of the juvenile and pre-adult gilthead seabream cultured under shaded conditions with improved diets were comparable to previous studies conducted in low intensity light. Among the improved diets tested the performance of the juvenile and pre-adult gilthead seabream in terms of FCR and SGR were better in shaded conditions. Based on the final weight gain, FCR and SGR in juvenile and pre-adult gilthead seabream the formulated improved diets were performed well at low light intensity.

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REPRODUCTIVE PERFORMANCE OF RAINBOW TROUT (*Oncorhynchus mykiss)* ASSOCIATED TO THE MHC-IIB GENE

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Introduction

Cryptic Female Choice (CFC) is a form of sexual selection operated by the female through chemical and physical mechanisms [1]. In some fish species, CFC has been associated to the Major Histocompatibility Complex (MHC). In the Atlantic salmon (*Salmo salar*), the presence of specific MHC alleles has been associated with a higher survival of the offspring [2]. In the rainbow trout (*Oncorhynchus mykiss*), the CFC phenomenon has not yet been demonstrated but there are indirect pieces of evidence for this process [3]. The present study aims to investigate the CFC phenomenon associated with the MHC-IIB gene in the rainbow trout by evaluating the reproductive performance of specific matings.

Materials and Methods

The selective matings and evaluation of physiological parameters were carried out at the "Azienda Agricola Canali Cavour" fish farm (Centallo, Italy). One hundred rainbow trout were selected based on their MHC-IIB gene haplotypes, obtained through amplification and direct sequencing[4]. Haplotypes were inferred through Bayesian statistics, considering 23 Single Nucleotide Polymorphisms (SNPs), using the software PHASE V.2.1.1. The rainbow trout were monitored to assess the reproductive status and to perform artificial reproductions. A total of 15 females (4 individuals with a haplotype 2/2, 4 with a 2/x and 6 with a haplotype x/y) and 12 males (4 per haplotype) were selected for the reproduction. Morpho-physiological parameters were recorded for each female: body weight and length, weight of the eggs produced, volume, density, and pH of the ovarian fluid. Matings were performed selecting individuals based on the three haplotypes according to a full-factorial design. The success of fertilization can be used to estimate the quality of a gamete. However, fertilization does not directly involve embryonic survival, since this should be assessed in different specific embryonic steps, such as the eyed stage, the hatching, and the yolk-sac resorption stage [5]. Therefore, reproductive performance rates were calculated following the literature [6]. Finally, from the data collected during the incubation period, the overall survival rates were estimated. For the collected parameters, the presence of differences among haplotypes was assessed with ANOVA or Kruskal-Wallis test. The Pairwise Wilcoxon Rank Sum Tests with Bonferroni correction was adopted as a post-hoc test. A generalized linear model was used for modelling the survival rates.

Table 1. Results of the GLM analysis on the survival rate.

Variables	β	Standard error	t-value	p-value
F 2/2 + M 2/x	0,04	0,09	0,46	0,65
F 2/2 + M x/y	0,01	0,09	1,14	0,26
F 2/x + M 2/2	-0,23	0,09	-2,51	< 0,05
F 2/x + M 2/x	-0,25	0,09	-2,75	< 0,01
F x/y + M 2/2	-0,050	0,09	-0,55	0,59
F x/y + M 2/x	-0,11	0,089	-1,24	0,22
F x/y + M x/y	-0,29	0,11	-2,68	< 0,01
Time	-0,09	0,02	-6,31	< 0,001
Single egg weight	5,53	3,42	1,62	0,11

(Continued on next page)

Results

Morpho-physiological parameters of the females did not differ significantly, except for the single egg weight and the relative fecundity. The value of the single egg weight was different between the haplotype 2/2 and the haplotype x/y ($p<0.05$). The parameters related to the matings (eggs and larvae) did not differ significantly, except for the deformed larvae rate ($p<0.01$) and the healthy larvae rate at the end of incubation ($p<0.05$). However, in both cases, the post-hoc test was not able to detect differences between groups, probably because of the low number of samples. From the GLM analysis on the trend of the survival rates it emerges that the type of mating and the time are the variables that significantly affect the survival (Tab. 1). The weight of the single egg is not significant and consequently has no effect on the survival rate.

Discussion

The lower egg weight did not affect negatively the reproduction performance of the female 2/2, since the mating with the 2/2 females showed, in average, higher reproductive rates compared with other matings. On the other side, some differences were noted in the larvae deformity rate ($p<0.01$) and in the rate of healthy larvae ($p<0.05$), however these differences were not confirmed by the post-hoc test due to a restrained sample size per category. The GLM analysis on the trend of the survival rates highlight that the type of mating and the time are the variables that significantly affect the survival. A significant effect emerges for the matings F 2/x + M 2/2, F 2/x + M 2/x and F x/y + M x/y, which compared with the mating F 2/2 + M 2/2 shows a lower survival. A lower survival rate is also observed for the F x/y + M 2/2 and F x/y + M 2/x matings, which however are not significant, as well as the F 2/2 + M 2/x and F 2/2 + M x/y which have a slight positive effect on survival.

Conclusions

This study aimed to evaluate the reproductive performance of rainbow trout associated with the MHC-IIB gene in relation to the CFC. Taken together, the results of the reproductive performance indicate a better output for the matings performed with 2/2 females. Furthermore, the GLM results indicate a greater survival during the incubation phase for the mating F 2/2 + M 2/2 if compared with other matings. Despite these encouraging findings, the results of this study are not able to confirm the presence of CFC in the rainbow trout (*O. mykiss*) associated with the mating among similar haplotypes (F 2/2 + M 2/2).

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COST ACTION 20106 “TOMORROW’S ‘WHEAT OF THE SEA’: ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE” (SEAWHEAT)

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Introduction

The COST (Cooperation in Science and Technology) Action CA 20106 – known as SeaWheat- aims to exploit seaweeds’ potential in Europe. Building on the successes of previous EU and pan-European algae projects, and thanks to the unique characteristics of the genus *Ulva* (Linnaeus, 1753), the Action identified these green algae as the most suitable organisms for biomass production and innovative blue biotech industries in European mariculture. *Ulva* (class Ulvophyceae) belongs to a genus inhabiting shallow marine and brackish waters and its species are ubiquitous throughout the world’s oceans, where they constitute an important primary producer.

Much of the knowledge on *Ulva,* generated in diverse scientific disciplines and communities, are not easily comparable, nor is it shared among scientists, stakeholders, end users and the public. This COST Action proposes an innovative conceptual pathway to address these issues, significantly improving knowledge on the biology of the most promising *Ulva* spp., capitalising on their economic potential, and exploring commercial applications in human nutrition, livestock nutrition, pharmaceutical industries and ecosystem services.

With seven different Working Groups (WGs), the Action has created a network of 190 participants belonging to 31 COST member states, including 20 SMEs (Small and Medium-sized Enterprises). The Action combines interdisciplinary approaches to the sustainable use of marine resources, encompassing all the facets of *Ulva* biology, ecology, aquaculture, engineering, economic and social sciences. In addition, it fulfils the current ‘Societal Challenges Priorities’ of European Horizon 2020 strategy for food security, and its application will contribute to the UN Sustainable Development Goals 14 (UNSDG) to conserve and sustainably exploit natural resources.

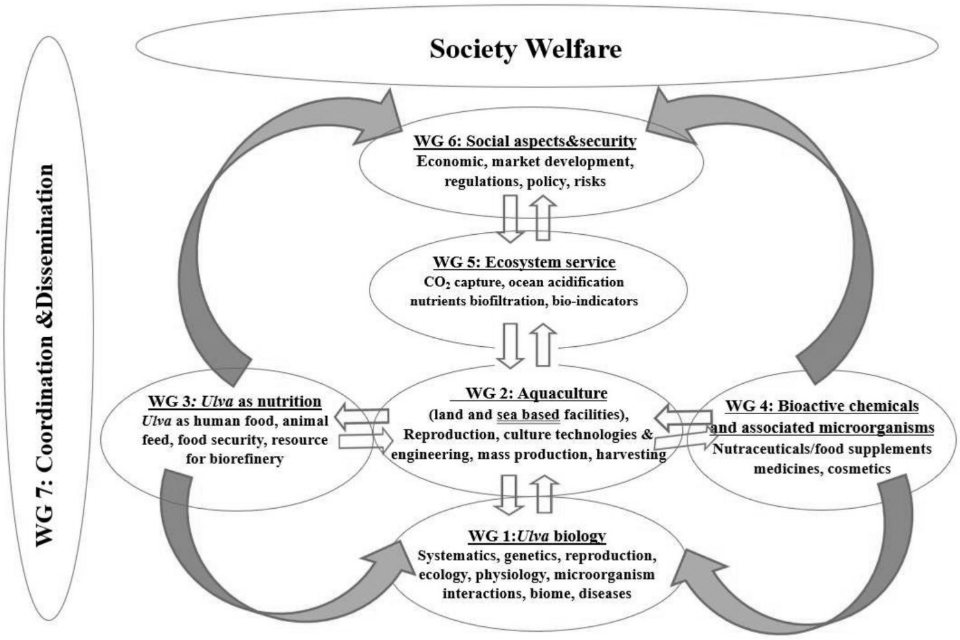

Fig. 1; Scheme of the Working Groups (WGs) in the COST Action and their interactions.

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Main Challenges

The main aim of the COST Action is to make a step-change towards a green economy based on *Ulva* mass production and utilisation within the European community and beyond. The ultimate goal is the development of *Ulva*-based blue-biotech industries and the utilisation of *Ulva* as a model organism in European algaculture.

Through the *Ulva* COST Action, the traditional European diet and taste will become acquainted with *Ulva* as a new, sustainable, and safe food item. Furthermore, thanks to its nutritional quality, the Action will promote *Ulva* inclusion in the European human diet and animal nutrition. Indeed, as an aquafeed ingredient, *Ulva* can facilitate a sustainable European aquaculture expansion. A high potential market also exists for new bioactive molecules that may provide innovative products in the nutraceutical and biomedical industries. Furthermore, *Ulva's* ecosystem services (provisioning, supporting, regulatory and economic), particularly nutrient recycling and removal and carbon fixation, will ensure environmental sustainability. However, large-scale production of *Ulva* is a prerequisite for making *Ulva*-derived compounds market-competitive.

The Action will bridge the scientific, regulatory and social practical knowledge gaps still existing in *Ulva,* paving the way to the commercial production of this seaweed in the blue-biotech industries.

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SUSTAINABLE ROADMPAS FOR AQUACULTURE: WHICH INDICATORS TO APPLY?

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Introduction

In the framework of the COASTAL project, two regions, the Charente (France) and the Danube Delta (Romania), are evaluating their aquaculture activities and opportunities with regard to their role in their further development. The EU is indicating the pathway towards a sustainable aquaculture with their Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030[1], for which sustainability of these activities should be assessable. For this, the EU has initiated the Blue Economy Sustainability Framework with Sustainability indicators for the Blue Economy (SIBE)[2], including aquaculture.

These indicators are covering the four dimensions of sustainability (economic, environmental, social and governance). For each dimension, a number of sustainable criteria were listed and different indicators established. There are 44 common indicators as well as 104 sector-specific indicators, divided over 12 subsectors. The SIBE wants indeed to obtain a cross-sectoral approach to the blue economy with flexibility for an application at different scales, levels and geographic zones. As the COASTAL project has an aquaculture focus in some of the project case studies, it could be of interest to cross-check the sustainable criteria listed by the SIBE with the in- and output generated through stakeholders interactions, dynamic models and their indicators and policy guidelines. The SIBE lists, aside the common indicators, three different subsectors of interest for aquaculture, namely “fish and shellfish harvesting”, “fish and shellfish processing” and “aquaculture”. While the French Charente region is looking to assure the production as well as the regional heritage of shellfish farming, the Romanian region is aiming to intensify its aquaculture (pondfarming and marine cagefarming) in the Danube Delta Biosphere reserve and its marine waters (Black Sea). Both region will have to deal with different challenges, but their further development should have to be sustainable. In that respect, indicators for assessing their negative impacts as well as their benefits in the different dimensions, have to be stipulated. This review will compare the different aspects of these aquaculture activities with the SIBE and help improve the sustainability indicators for aquaculture.

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INNOVATION IN THE NORWEGIAN AQUACULTURE INUSTRY

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Introduction

Global aquaculture production has grown very rapidly in recent decades. This is largely due to a number of innovations that has increased the control with the production process and competitiveness. These innovations come in a number of forms from radical new concepts to knowledge adaption from the terrestrial food production system. While there exist a number of studies investigating the impact of specific innovations, there are few studies that takes a larger perspective on how innovations over time impacts an aquaculture industry or the innovation system that support these innovations.

Materials and methods

Based on secondary data gathered from media, industry and government reports, as well as previous research, in this paper we review the innovation process in Norwegian salmon aquaculture industry from its infancy in 1970 until present. Of particular interest is the increasing complexity of the industry, and how most innovations are conducted by suppliers and not the aquaculture producers themselves.

Results

The success of the Norwegian salmon aquaculture industry over the last decades has largely resulted from innovations across the aquaculture value chain such as genetics (breeding), fish feed, feeding equipment, vaccines, information technology and cages. Innovations have contributed greatly to increased growth rates for salmon, lower mortality, higher product quality and lower production costs (Asche et al., 2013).

As in most aquaculture industries, the original Norwegian salmon farmer was an entrepreneur, who was also a "Jack of All Trades" in that the farmer had to do everything involved from building the farm, raise the fingerlings, mix the feed and harvest the fish. As the industry grew, there has been a dynamic process of innovations that has enhanced productivity and increased the control with the production process. This has largely been conducted by new supplier industries, where the specialized suppliers identified the growing industry as a market, and competition between suppliers lead to innovations giving better inputs at a lower cost (Tveteras and Heshmati, 2002; Asche, 2008). Today, there are specialized suppliers for a wide range of equipment such as (but not limited to) sensors and control systems as well services such as veterinary tests, net cleaning and research. As such, an increasing part of the employment associated with the industry is in different types of suppliers.

A common thread is that the size of everything has increased in salmon aquaculture, the production, the fish, the pens, the farms, the feed barges, the well-boats, the processing plants and the firms. This suggest that innovations are also important for creating and allowing economies of scale to be exploited. Innovations have generally been scale-biased or scale-increasing, and through the value chain from smolt production via sea transportation and grow-out farming to primary processing the optimal economic scale has increased. Not unexpectedly, many of the most radical innovations occurred early in the history of the industry, and later innovations are mostly smaller process or product innovations which in sum provides a powerful gradual process. However, there still occur radical innovation as exemplified by offshore and land-based farming. It is also interesting that public incentives in combination with the regulatory system is facilitating such innovations.

The innovations provide more technological opportunities for salmon aquaculture than we have ever seen before. Innovations in open and closed production systems from land-based or onshore to offshore ocean allow for several new value chain configurations. These new production technologies have the potential to both reduce firms' internal production costs, as well as reduce the external costs of environmental emissions, diseases and Salmon lice. As of now, no-one knows what is going to be the production concepts used in salmon farming in the future, or even whether there will be one dominating one or several parallel concepts used. However, it is highly interesting that the basic production technology, open sea pens, are being challenged, and that all the new concepts increase the control with the production process and thereby the potential for further innovation.

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Conclusion

Although our research is focused on innovations in the Norwegian aquaculture industry, the insights are also of general interest in global aquaculture as salmon is among the species with the most advanced production technologies, but also a species where innovations are adapted to other species also in very different production systems. Furthermore, in this paper, we have solely focused on the production part of the supply chain. However, as consumers in general only care about the price they are paying, innovations in the supply chain are equally important for the competitiveness of any industry. Previous research has shown that the supply chain for salmon is highly competitive in relation to most other seafood species (see Asche and Smith 2018). Compared to other more advanced (especially terrestrial) production systems such as poultry (e.g., chicken) however, the supply chains for salmon is still lagging behind in many dimensions (Asche et al., 2018). Hence, there is still significant scope for innovations also down-streams in the supply chain. This includes not only logistics and product development, but also perceptions of the species. This is particularly important for salmon since there is a number of issues with respect to its environmental sustainability that can at least partly be addressed by certification (Osmundsen et al., 2020).

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PRESENCE OF THE OSTREID HERPSVIRUS IN PORTUGUESE OYSTER, *Crassostrea angulata,* AND IN PACIFIC OYSTER, *Crassostrea gigas*, IN PORTUGAL

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Introduction

Several pathogenic agents can cause lesions and diseases in bivalve molluscs both in wild and in production areas. *Ostreid herpesvirus* (OsHV-1 and OsHV -1 *μ*var) has been the cause of important mortalities in Japanese oyster (Crassostrea gigas), in several countries, including in Portugal since 2012. The lesions are not pathognomic of that virus and include, namely, discoloration of the digestive gland, presence of vacuoles in the cytoplasm of epithelia of the digestive gland diverticula and necrotic lesions in several tissues, mainly in gills epithelium and connective tissue. Furthermore, hemocitosis and intracytoplasmatic inclusion bodies can be observed in most affected tissues.

Materials and Methods

The presence of OsHV-1 and OsHV-1 *μ*var in Portuguese oyster and Japanese oyster were surveyed from January 2021 until May 2022. Oysters (adults) were sampled from three different sites of the Portuguese coast (water temperature: 18-20ºC; salinity: 30-34 ppm). Portuguese oyster from Sado estuary (n=74) and Japanese oyster from Formosa lagoon (n=63) and Aveiro lagoon (n=136).

For the detection of this pathogen we followed, since the first time thepathogen was detected in Portuguese waters (2011), the protocol set out in COMMISSION REGULATION (EU) No 175/2010 of 2 March 2010, Part B of Annex I for the DNA extraction method and PCR analysis. The tissue samples for histopatholgy were fixed in Davidson's fixative for 48h,dehydration, embedding in paraffin and cutting with a microtome in sections with3-5 *μ*m thick, than stained with Hematoxylin and Eosin (H&E).

Results

The histopathological examination of both oysters species revealed the presence of *Trichodina sp*. ciliates in gills and mantle epithelium and *Ancistrocoma sp*. in the digestive gland tubules and in the connective tissue, both with a moderate prevalence. The copepod *Mytilicola sp*. was observed in the intestine lumen of both oyster species. In both populations, hemocytosis was observed in connective tissue, edema and metaplasia in digestive gland and tissues necrosis. In the population from Mira estuary the prevalence of these lesions was slightly higher, except for individuals with metaplasia.

The lesions observed in the epithelium of the diverticula of the digestive gland, the hemocytic infiltration and necrosis can be related with different pathogenic agents.

In all populations analyzed in this study the presence of the virus was not identified.

Discussion and conclusion

The first mortality outbreak observed in Portugal, occurred in populations of Pacific oyster, *Crassostrea gigas*, related with the presence of a herpes-like virus,*Ostreid Herpsvirus* (OsHV-1), reported during the summer of 2012 in an offshore long line culture systems, settled near S. Vicente Cape, in south coast of Portugal.Associated with high mortality rates, was detected for the first time in 2011, the presence of the µvar genotype of OsHV-1 in *C. angulata* produced from a broodstock collected in Sado River and then transferred to the Formosa Lagoon (Batista et al., 2015). This disease reached the highest impact in oyster production during 2017, when mortalities rates between 60 and 100% where registered in Alvor (Algarve) and Aveiro lagoons. Since then, reports of massive mortalities have been declining.

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Environmental factors, as well as managing culture practices, namely hight densities in oyster tables, and intensive handling of the animals during critical environmental conditions, can contribute to increase the pathogenicity and the spread capacity of this pathogenic and of those that are opportunistic. However, biosecurity measures are fundamental to prevent the spread of the disease, namely control of the transport of the animals and of the equipment from different countries and regions and quarantine. Also, regular laboratory control, the identification and the characterization of pathologic processes in oysters are important measures for sanitary control. All together, these measures and the collaboration of the producers have been contributing to the control and spread of *Ostreid Herpsvirus* in Portugal.

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COMPARATIVE ANALYSES OF TWO OYSTER FARMING SYSTEMS FOR *Ostrea edulis* AND *Magallana gigas* IN A BRACKISH BASIN OF VALLI DI COMACCHIO (NORTHERN ITALY)

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Introduction

The demand of farmed oysters in Italy is increasing since 2010 (Lopes *et al.*, 2020), due to better quality of shape and organoleptic quality of meat compared to wild specimens. The research of new cultivation areas and farming systems are fundamental to answer the request.

Although the Pacific cupped oyster (*Magallana gigas*) is the most widely cultured species, the European flat oyster (*Ostrea edulis*) could also represent a good chance of development especially in areas where the parasites *Bonamia ostreae* and *Marteilia refringens* are not endemic.

The aim of this study is to introduce the "off-bottom" oyster cultivation method in Valli di Comacchio (Northern Italy), that are brackish basins mainly dedicated to fish farming. The channels linking the basins to the Adriatic Sea are highly productive and well established in the cultivation of Manila clams (*Ruditapes philippinarum*) and they also hold large benches of *M. gigas*, while *O. edulis* represents an occasional catch.

Methodology

The study was conducted in Valle Sottolido, a basin of 60 ha and mean depth of 1.5m. The sea water arrives to the basin through a 4,2 km channel and the water flow is influenced by tides. Water characteristics were investigated using data loggers (temperature, dissolved oxygen, salinity), while the chlorophyll-a concentration was measured weekly by spectrophotometry after extraction with acetone (Lorenzen, 1967), during the period January – July 2022.

O. edulis and triploid *M. gigas* spat were placed into floating cages of FlipFarm™ system, stocked at 100 individuals cage^{-1} and they were nested within two daily immersion time: 18h and 24h, in order to test also the effect of immersion time on survival, growth, shell composition and meat quality.

To evaluate differences in chemical and bromatological composition between *O. edulis* and *M. gigas*, also related to both farming techniques, the oysters were opened, and the soft body was removed from the shell. The soft body was homogenized and lyophilized for lipids, proteins, ashes, and carbohydrates determination. The element composition of shells (previously grounded) of both species, was determined by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES). The concentration of elements Ca, Sr, Fe, Mg, Si, Al and S was calculated.

Results

M. gigas farmed with 24h of daily immersion time grew till an early marketable size in 180 days. Oysters of the same species but farmed with 18h of daily immersion time had a slower growth. Both treatments resulted in survival above 90%. *O. edulis* showed a slower growth than *M. gigas* and more mortality, in particular on the 18h immersion time treated batch.

The higher growth rate was found during the month of April 2022, when average day temperature ranged from 13,9°C and 21,3°C and Chl-a concentration was not low than 2,314μg/l.

Bromatological composition of *O. edulis* and *M. gigas*, did not show any quantitative nor qualitative differences within the two cultivation methods. However, a higher percentage of total lipids was detected in *M.gigas* compared to *O.edulis*. In detail, *M. gigas* showed a higher percentage of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) for both cultivation methods. The elements concentrations of the shells, obtained by ICP-OES, differed between the two cultivation methods and between the species. In particular, the shells of animals grown for 24h immersion contained higher concentration of each element compared to oysters grown for 18h immersion, except for Ca, that did not show any differences.

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SPONGES AS EMERGING BY-PRODUCT OF INTEGRATED MULTITROPHIC AQUACULTURE (IMTA): AN OVERVIEW IN THE LIGHT OF REMEDIA LIFE IMTA SYSTEM

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Introduction

Coastal fish farming plants can have a significant environmental impact due to the release of catabolites by farmed organisms and feed residues, causing an overall increase in organic matter and potentially pathogenic or antibiotic resistant bacterial load in the water body. In this sense, Integrated MultiTrophic Aquaculture (IMTA), the practice that combines the cultivation of fed aquaculture species with other organisms able to extract organic and/or inorganic substances from seawater, represents an eco-friendly alternative to the monoculture. IMTAs allow water quality improvement, promise high economic return and social suitability also implying an improvement in human welfare and in the quality of ecosystem services provided by the marine environment. Among extractive organisms, filter-feeders and particularly Porifera seem the most promising although they are still little known or underexploited in these activities. Their environmental and economic potential and thus the interest in these organisms is increasing as new sustainable and profitable applications for the biomass obtained from these systems are developed.

Bioremediation, the basis of sponge production

One of the main advantages of filter-feeding organisms such as Porifera is precisely that their energy source is obtained by highly efficient filtration of organic particles between 0.1-50 μm, such as dissolved and particulate organic particles, heterotrophic bacteria and eukaryotes, phytoplankton and even viruses (Simpson, 1984; Riisgård & Larsen, 1995; Hadas et al., 2006), so that their mere growth leads to bioremediation of the surrounding environment. Similarly, being filter-feeding organisms, they are constantly exposed to contamination of the water column, which makes them target organisms as bioremediators or bioindicators due to their (and their microsymbionts') ability to accumulate metallic and organic pollutants (e.g. Bauvais et al., 2015; Gentric et al., 2015).

Past and present concern

The trade of sea sponges dates back hundreds of years, when they were collected for cosmetic use known as "bath sponges"; today, in the Mediterranean basin countries such as Italy, Greece, Croatia, Cyprus or Tunisia still maintain this economic sector (e.g. Spugnificio Incorvaia, Italy; Spuga2, Croatia). However, the unregulated overexploitation of this resource in the past has caused the cultivation of sponges to attract increasing attention over the years, in addition to the shift in commercial interest in their production from rearing of bath sponges to their cultivation for the production of secondary metabolites (Biertwirth et al., 2022).

A marine bioactive compound factory

The first marine origin drugs to reach clinical trials were extracted from a Caribbean sponge around seven decades ago (Bergmann & Feeney, 1951); since then, the number of these compounds has only increased and together with cnidarians, they are the most prominent organisms for their discovery, exceeding 200 per year (Carroll et al., 2019). These compounds are synthesized not only by sponges, but also by their symbiont microorganisms (Proksch et al., 2002). They have a wide range of applications: from anti-tumour, antiviral and antimicrobial in human medicine up to antimicrobial and antifouling activities in the aquaculture industry or biomaterial production in different sectors (Rotter et al., 2021; Varijakzhan et al., 2021). However, obtaining the needed biomass involves a considerable challenge, as natural populations are unable to supply this market in a sustainable manner. Therefore, co-culture strategies such as IMTA appear as a possible solution, as the natural environment provides the optimal conditions (even for endosymbiont microorganisms) that *ex-situ* can be costly and difficult to maintain.

From the sea to the tank

Another economic potential of marine sponges is their sale for recreational aquaria, where, due to their diverse colours and shapes, they are particularly attractive. There are companies dedicated to their trade (mainly in the US), and their prices vary from tens to hundreds of euros per individual depending on species and size (e.g. RUSALTY, US). In addition, there is a growing interest in the sponge specimens supplies as food for certain spongivorous fishes. In this sense, facilities such as IMTA systems could provide a suitable environment for the growth and sustainable production of local species.

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The challenge in marine sponges' restoration

Combining production for human consumption with restorative aspects is a recent and arising approach (Giangrande et al., 2021). Wisely planned IMTA could be reasonably proposed for environmental restoration and conservation purposes. Such plants combine bioremediation with biodiversity increase. In the case of sponges, the biomass obtained can be valorized for transplantation or restocking of threatened or vulnerable species up to benthic habitat restoration.

Conclusions

The production of biomass from non-edible species in properly designed IMTAs such as marine sponges with important profitable applications implies the use of new resources that promotes a blue circular economy. In this sense, Remedia Life IMTA system represents an eco-friendly sponge biomass supply not only for economic interests such as green extraction of bioactive molecules and aquariology (ornamental species, food or shelter for fishes) but also for environmental welfare as bioremediators or restoration purposes that increase ecosystem biodiversity.

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CO-PRODUCTION OF KNOWLEDGE FOR A DECISION SUPPORT TOOL FOR AQUACULTURE AND COASTAL MANAGEMENT DEVELOPMENT IN NORWAY

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Introduction

There are several challenges currently limiting the production growth of conventional aquaculture in open net cages in Norway including sea lice, emissions, and escaped salmon. New production systems may allow for growth though, by allowing for production at more exposed localities, in land-based farms and even in semi-closed or closed floating cages. These systems can battle some of the challenges faced by conventional aquaculture still though, and each also have other challenges connected to their respective systems, both on the technical and on the regulatory and management side.

For the purposes of this study, we co-develop a decision support tool in close collaboration with stakeholders, based on conceptual mental models created with Fuzzy Cognitive mapping (FCM) in the software Mental Modeler (Gray; Kosko, 1986). Groups of experts and stakeholders within a given area decided on relevant variables and their connections within a specific field, which formed the basis of the mental models. The decision support tool allowed us to develop stakeholder driven future scenarios aimed at countering the identified sustainability issues by testing a broad range of management options, to assess trade-offs and establish mitigation and adaptations needs. These scenarios can theoretically verify if impacts of different measures implemented work as intended or to identify where and which measures are needed to regulate or manage a change in a system. It is therefore the aim that the tool can aid policy makers, industry, stakeholders, and researchers alike with stakeholder driven data that give legitimacy when making critical choices in terms of what to prioritize given different weighing of SDGs for example.

We have included two case studies to test the decision support tool. These are mental models on the new aquaculture production systems in Norway, and on coastal-rural interaction due to the land-sea utilization of different industries along the coastline in Norway. The aim is to make a generic tool that can run scenarios with different datasets from diverse socio-ecological settings using Fuzzy Cognitive Mapping.

Methods

The input dataset referred to as the mental model, was created with FCM in Mental Modeler. Several representatives and experts for the aquaculture industry have collectively participated and agreed on which variables and their connections are important for the specific case study. These variables include amongst others “Optimal feeding”, “Oxygen levels”, “Regulations, “Acceptance of local communities” and more. The variables are connected to the other variables that they directly influence. These connections have been quantified on a scale between 0 and ±1. A negative value indicates that if one variable increases, the “negative” connection decreases, or opposite with a positive value, then an increase would lead to an increase. These values have been determined and later validated by the expert panel from the stakeholder groups.

The decision support tool has been created in R (R Core Team, 2020), which is a free software and programming language specialized for statistical computing, data mining and graphics. We are using the existing functionality in R related to FCM, such as the FCM package (Dikopoulou & Papageorgiou, 2017) for model simulations.

The scenarios are visualized in the decision support tool, where each variable is presented in the same figure. In this figure, the value of each variable changes over the iterations of the model, until the model has reached one of the three following states: 1) Equilibrium, 2) A cycle behaviour or 3) Chaotic behaviour. These states are presented by the function as “convergence” since they become fixed points. These graphs represent the storylines of the scenarios, that are determined by the initial value of variables set by the user, and the mental model defined by the experts. Therefore, these scenarios are modelled based on the stakeholders’ perception of the system if there is a change to the current system. The decision support tool will be verified and calibrated further by the experts from the specific case studies.

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Results & Discussion

The decision support tool allows the user to change the values of one or several variables at the time. The tool then models a scenario based on the choice of the user. The results are visualized graphically where the user can see the development of each variable over the iterations.

To illustrate the area of use and possibility the decision support tool gives, we have selected some examples. If, in the new aquaculture production system's case, "Acceptance of local communities to a new production system" was increased to 1 (the highest possible value, representing "complete acceptance"), the decision support system would see a similar increase in production. This, however, would also trigger a demand for a larger workforce, which could again affect the initial "Acceptance" variable. Another example from the coastal-rural interaction is that if "Infrastructure" improved in the North of Norway, "Transportation" would increase, which would lead to increased "Production" from the industry in the municipalities. On the other hand, improved "Infrastructure" would also lead to increased "Emissions".

The scenarios are heavily dependent on the mental model used, which again is based on the inputs of the participants. The scenarios can identify policy options, business strategies and practices for policy makers, industry actors and other stakeholders that support the sustainability of the aquaculture sector. The power of the decision support tool is that it can uncover connections and repercussions that are not apparent. Another benefit is that it is co-produced with the stakeholders themselves, who know the system in ways that scientists in isolation may not be able to represent. The tool does allow the users to create scenarios and predict how the rest of the system reacts, providing crucial inputs for both production planning, policy making and resource management.

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TWO-PHASE FLOW MODELING VIA COMPUTATIONAL FLUID DYNAMICS (CFD) STUDIES ON A CASE STUDY: MOVING BED BIOMEDIA REACTOR (MBBR) 1000

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Total Ammonia Nitrogen (TAN), which occurs due to various reasons especially fish defecation and death, is crucial for life and bring about death of living creatures. Therefore, it is very important to reduce the amount of TAN in water and it is called as Nitrification process.

In order to carry out the nitrification process in the best way, the bacteria must be optimally provided in the environmental conditions of the habitat. The parameters that ensure these are: 1) biomedia surface, 2) pH of water in unit, 3) amount of oxygen in the air, and 4) optimum two-phase flow of water-air flow. There are two ways to remove TAN in fish farming and industrial aquariums. One of them is Trickle filter and the other is Moving Bed Biomedia Reactor (MBBR). It can be said for these two systems that provide advantages over each other, but to sum up; the MBBR is more user-friendly and more advantageous in that it provides higher removal rate of TAN in the same size. By giving the air taken from the blower into the water, a double phase flow will be provided inside the MBBR unit. Modeling this is important in terms of the accuracy of the intermediate and main parameters.

The aim of this study is to structure the project according to the parameters that need to be optimized above. The methods used consist of mathematical modeling according to the amount of TAN determined on a project basis, MBBR unit sizing, biomedia diversity and mixing ratio determination, and finite element analysis with ANSYS CFX® to model two-phase flow.

REDUCING RISK IN OPERATION OF EXPOSED HIGH VOLUME, ADVANCED FISH FARMS - HOW TO MAINTAIN SAFETY OF ASSET, PERSONNEL, MAINTAIN FISH WELFARE AND PREVENT FISH ESCAPE FOR OFFSHORE FISH FARMS

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INTRODUCTION

It is a growing trend that fish farms are moved out of sheltered, near-shore areas and moved to locations further offshore. There are several advantages applying this solution, Better fish welfare, less conflicts with interests in the ocean space as well as possibility to scale up output volume without compromising environmental sustainability are some of them. As the world's population grows, the options for producing enough food are becoming limited. The oceans offer a vast opportunity to meet this demand with sustainable, safe and efficient offshore fish farming.

Adding safety to offshore fish farming projects is mainly ensured by providing uniformity, transparency, and predictability and thereby reducing project risk. We need to be ensure that facilities for aquaculture can handle harsh environment and still contain the fish safely.

MAIN APPROACH

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

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

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

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

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

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

CONCLUSION

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

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

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Photo Nordlaks Oppdrett

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BY-PRODUCTS FROM CRAB SHELLS INCREASE FEED INTAKE, GROWTH, SKIN QUALITY AND HEALTH IN ATLANTIC SALMON *Salmo salar* SMOLT AFTER SEAWATER TRANSFER

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Introduction and aim

In the first period after seawater transfer it is essential to use high quality feed ingredients that can stimulate appetite and growth of salmon and thereby improve quality and robustness of fish. High production losses of salmon are annually reported in the period after seawater transfer due to development of wounds and injuries that makes the fish prone to infections and fungal attacks. Some of the free amino acids in fish protein hydrolysates are known to affect smell and taste perception in fish, and hydrolysates produced from marine by-products have demonstrated effective feed attractant properties in salmon. The aim of this project was to document impacts of crab hydrolysate and crab meal ingredients produced from crab shells on feed intake, growth and skin quality in Atlantic salmon when supplied as an additive in salmon feed after seawater transfer.

Material and Methods

The crab products were added at 2 dietary levels (1.5%, 3.0%), mainly by replacing crab protein with fishmeal protein, but also with small corrections with plant proteins. The crab hydrolysate contained 39% protein and replaced 0.9 and 1.8% of the protein, respectively, while crab meal contained 16% protein and replaced 0.35 and 0.69% protein. Both crab products were added by top-coating on extruded feeds. A feeding trial with Atlantic salmon in the first 6 weeks period after transfer was performed. Results with respect to feed intake, growth and dietary impacts on skin quality was recorded by visual and histological analyses and by transcriptional profiling and compared to results obtained by feeding a similar formulated control diet without crab products.

Results

Crab hydrolysate used as an additive in feed for salmon and fed for 6 weeks after seawater transfer, stimulated feed intake and growth more effectively than crab meal added at the same dietary levels, and improved feed intake and growth significantly as compared to control fish ($P < 0.05$). A linear relation between feed intake and growth was found, indicative of efficient nutrient utilization in all dietary groups, as confirmed by no or small differences in nutrient digestibility. Feed intake responses equivalent to 3 % of fish biomass was recorded after 5 and 10 days in fish fed crab hydrolysate and crab meal diets (only Crab M1), respectively, and after 15 days of feeding in fish fed the control diet. The variable time of onset of feeding after seawater transfer explain most of the variation in fish growth. Feed intake responses equivalent to 3 % of initial biomass was recorded after 5, 10 and 15 days of feeding in fish fed crab hydrolysate, crab meal (Crab M1) and control diets, respectively.

Salmon fed crab hydrolysates gained 78 - 83 % of their initial weight and showed efficient growth rates; mean SGR = 1.44 ± 0.09 % during 6 weeks of feeding after transfer, which is significantly higher than in fish fed the control diet; mean SGR = 0.95 ± 0.06 % ($P < 0,05$). Salmon fed crab meals gained 59 - 60 % of their initial weight, and the growth in this group was not significantly different from control fish ($P > 0.05$).

Histological examination of the dorsal skin (Aiforia) showed thicker skin in the dermal layer, and increased area and number of mucus-producing cells in the epidermal layer. Significantly lower dark pigmentation was found in the skin of fish fed crab hydrolysate diet compared to fish fed the control diet ($P <0.05$). Dark skin pigmentation is regarded a non-specific reaction typically found in fish exposed to frequent handling, stress, and diseases.

A visual assessment of the dorsal skin quality (FishWell Operational Welfare Indicator scores) that included fish shell losses, wounds, and bleeding, confirmed better skin quality in fish fed with crab hydrolysate. At the same time, anti-inflammatory properties were detected in the skin of salmon fed with crab hydrolysate, where several well-known genetic markers (serum amyloid A-5 protein, matrix metalloproteinase 13-2, 9-1, 9-2) and other less described follow up markers of acute inflammation were significantly lower expressed as compared to fish fed the control diet without hydrolysate ($P <0.05$). Histopathological changes in the upper skin layer (oedema, altered morphology) consistent with early infectious stages of *Moritella viscosa*, a fish pathogen associated with wounds and mortality in salmon farming, was observed. The significance of the anti-inflammatory action of crab hydrolysate shown during the early infectious stage in fish, is not entirely understood and warrants confirmation.

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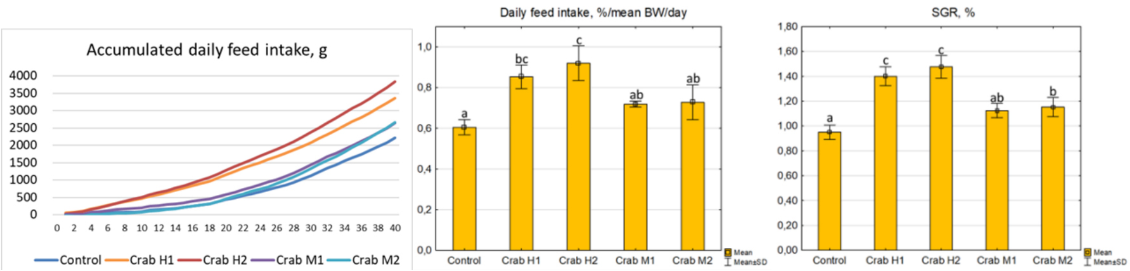


Figure 1 Accumulated (g) and daily (%/mean bodyweight (BW)/day) feed intakes and daily specific growth rates (SGR, %) in Atlantic salmon after 6 weeks of feeding post transfer. Mean values ± STD(x) are given. D1: Control; D2: Crab hydrolysate (H1); D3: Crab hydrolysate (H2); D4: Crab meal (M1), D5: Crab meal (M2)

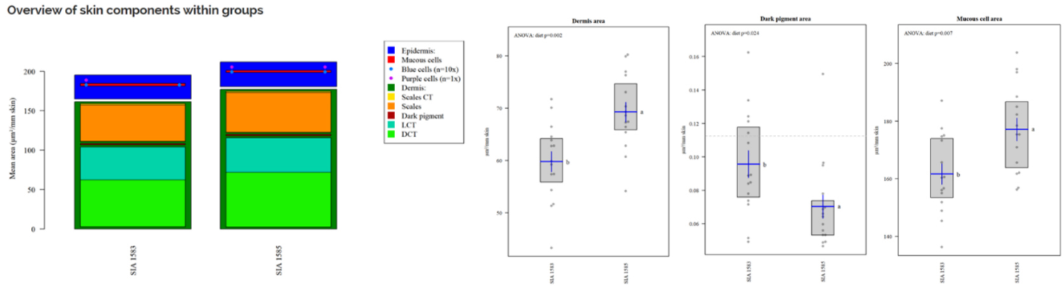
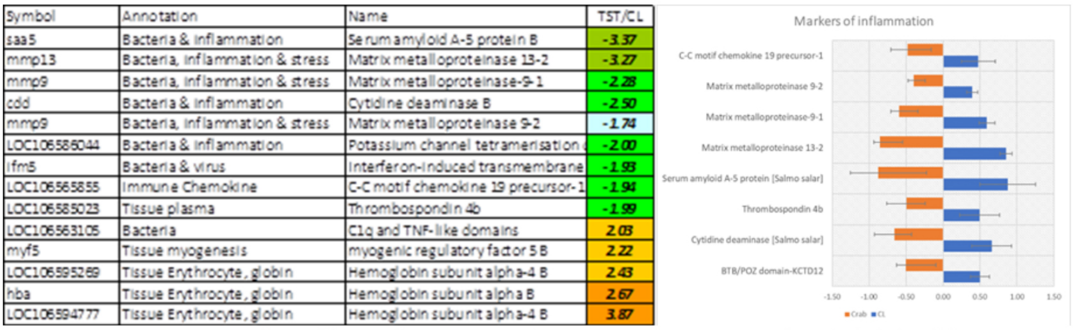


Figure 3 Overview of variable skin components of fish fed control diet (SIA 1583) and Crab hydrolysate H2 diet (SIA 1585). Dermis area was significantly higher, dark pigment area significant lower, and mucous *cell area and number of N cells per mm skin in fish significant higher in fish fed Crab H2 compared to control fish.*



Symbol	Annotation	Name	TST/CL
saa5	Bacteria & inflammation	Serum amyloid A-5 protein B	-3.37
mmp13	Bacteria, inflammation & stress	Matrix metalloproteinase 13-2	-3.27
mmp9	Bacteria, inflammation & stress	Matrix metalloproteinase-9-1	-2.28
cdd	Bacteria & inflammation	Cytidine deaminase B	-2.50
mmp9	Bacteria, inflammation & stress	Matrix metalloproteinase 9-2	-1.74
LOC106586044	Bacteria & inflammation	Potassium channel tetramerisation	-2.00
ifm5	Bacteria & virus	Interferon-induced transmembrane	-1.93
LOC106565855	Immune Chemokine	C-C motif chemokine 19 precursor-1	-1.94
LOC106585023	Tissue plasma	Thrombospondin 4b	-1.99
LOC106563105	Bacteria	C1q and TNF-like domains	2.03
myf5	Tissue myogenesis	myogenic regulatory factor 5 B	2.22
LOC106595269	Tissue Erythrocyte, globin	Hemoglobin subunit alpha-4 B	2.43
hba	Tissue Erythrocyte, globin	Hemoglobin subunit alpha B	2.67
LOC106594777	Tissue Erythrocyte, globin	Hemoglobin subunit alpha-4 B	3.87

Figure 1 Transcriptional profiling of genetic markers strongly associated with fish health. Data are presented as test group (Crab hydrolysate H2) relative to control folds (CL).

Conclusions

The improved feed intake and growth-stimulating properties of the crab products, in particular the crab hydrolysate, is mainly explained by effective release of appetite-stimulating water-soluble compounds into the seawater. The results suggest positive impacts of the crab hydrolysate on skin quality, and strong anti-inflammatory properties of significance for quality, health, and robustness of salmon. The collagen rich dermal skin layer in salmon grows in line with fish growth, and the thicker dermal skin layer observed may be explained by higher body weight of fish fed the crab hydrolysate. The positive impacts on mucus producing cells and the strong anti-inflammatory properties measured in fish fed the crab hydrolysate, however, appear to be of dietary origin. Overall results suggest positive quality and health promoting impacts of crab hydrolysate produced from crab by-products, that may contribute to increase the utilization of available resources. The crab meal was less efficient as feed attractant in salmon but has previously been reported as a valuable high ash, growth stimulating ingredient in cod feed, that may also contribute to sustainable aquaculture production.

THE EFFECTS OF MELATONIN IN PRESERVING GILTHEAD SEABREAM (*Sparus aurata*) SPERMATOZOA: PRELIMINARY RESULTS

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Introduction

Many species of fish can be benefited from the use of cryopreservation as a tool for artificial reproduction in genetic development programmes (Cabrita et al., 2010; Magnotti et al., 2018; Martínez-Páramo et al., 2017). During cryopreservation, the quality and viability of the sperm shows significant decline , mainly due to the significant increase in the production of reactive oxygen species (ROS) and the oxidative metabolism alteration during the freezing -thawing process (Marques et al., 2019). Besides its several well-known physiologic roles, melatonin has a significant antioxidant potential through free radical scavenging properties (Meng et al., 2017). Melatonin and its metabolites, shows protective action based on their direct and indirect antioxidant properties (Reiter et al., 2017). The main aim of this experiment was to examine the protective role of melatonin, tested on gilthead seabream spermatozoa during in vitro storage at 4°C and -196°C (liquid nitrogen) respectively.

Materials and Methods

The current experiment was carried out during the reproductive period of gilthead seabream (November to May in Greece). A total of 4 samplings were carried out and in each one 2 pools of four individuals were formed. The sperm collection was done individually for each fish and for the formation of the pools only sperm with a total motility >80% were used. Samples were diluted 1:3 in the extender 1% NaCl w/v (300 mOsm/kg) plus 1% BSA w/v. In the control group was added 5% DMSO v/v. In the treatment groups were tested 0.5mM, 1mM, 1.5mM and 2mM melatonin concentrations. Motility parameters were assessed with CASA (Computer Assisted Sperm Analysis) Hamilton Thorne CEROS 14.0 Version analyzer, under a Zeiss-Scope A1 contrast phase optical microscope, using an objective lens with 10× magnification. Specifically, were determined Total Motility (%) and Progressive Motility (%), Path Velocity (VAP, μm/s), Straight Line Velocity (VSL, μm/s), Curvilinear Velocity (VCL, μm/s), Straightness (STR, %) and Linearity (LIN, %) and the Velocity Distribution (Rapid, Medium, Slow and Static spermatozoa, %). In the experiment during short-term cold storage at 4°C the previous parameters were evaluated at 24h, 48h and 72h post dilution. The kinematic parameters of the spermatozoa were statistically analyzed with the methodological framework of General Linear Models Anova (GLM: General Linear Models) for all sperm collections as a whole and for each separate one, using the static program Minitab 17, USA. The significance level was set at 5% ($P<0.05$) for all parameters.

Results

In the experiment during freezing storage at -196°C, there were no significant differences between melatonin treatments and control group. The fresh sperm presented the best values of total motility and velocity parameters. In the experiment cooling storage at 4°C, there were significant differences between melatonin treatment groups and control and fresh sperm group in Total Motility (%), Static cells (%) and Path Velocity (VAP, μm/s) at all three times of evaluation (24h, 48h and 72h). Treatment with melatonin presented the best values of these parameters.

Discussion

From the above results, we can conclude that during the short-term cold storage of gilthead seabream sperm at 4°C, the supplementation of melatonin into the cooling extender, significantly improves the quality of sperm in terms of total motility. Spermatozoa remains motile for a longer period of storage compared to the fresh sperm and the control group. In contrast, during freezing sperm storage in liquid nitrogen no statistically significant differences were observed in any of the four melatonin concentrations tested, compared to fresh sperm and the control group. Further research is needed to find out the beneficial melatonin concentration into the freezing extenders during storage of *Sparus aurata* sperm at -196°C (Alevra et al., 2022).

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INSIGHTS FROM REAL-TIME MONITORING OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) SEA CAGE MARICULTURE

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Introduction

The fish farming industry needs instruments that can monitor in real time fish health and welfare objectively, without disturbing the fish or interfering with the daily management. In this context, precision livestock farming is gaining increased attention to enhance animal welfare, but also to enhance production and environmental sustainability (Halachmi et al., 2019; Brijs et al., 2021). In this study, we monitored European sea bass (*Dicentrarchus labrax*) implanted with acoustic transmitter which is measuring fish depth and swimming activity, a proxy of energy expenditure at the KEFISH farm at Argostoli (Greece) over ~4 months. In addition, environmental sensors were deployed in the farm cages to monitor environmental variables (temperature, oxygen concentration, salinity and turbidity). The aim of this work was to investigate the possibility to model key performances indicators (KPIs; i.e., growth performance and fish mortality) of sea bass using the physiological and environmental parameters recorded using a wireless sensors system, and so to investigate if the use of such sensors may help management procedure, enhance fish welfare and production.

Materials and methods

The environmental and physiological sensors were deployed in the KEFISH farm for a monitoring period of 134 days (from the 09.20.2021 to 01.31.2022). Over the experiment duration, morphometrics measurements were carried out by the farmer when fishing (n=7 sampling times) allowing the estimation of the weight gained by fish per day over the trial. Mortality events (count of dead fish) were also recorded by the farmer every day. Physiological sensors used in the study were acoustic accelerometer tags with 'tailbeat mode' algorithm, allowing to measure fish acceleration (m s^{-2}), which is a proxy of energy expenditure (Alfonso et al., 2021). Five environmental sensors were installed in the cage (Figure 1). Among the five sensors, we installed three dissolved oxygen (DO) sensors, one turbidity sensor and one salinity sensor. In this trial, we unfortunately faced issues with the outputs of both turbidity and salinity sensors. Thus, being not satisfied of the outputs of these parameters, they have been not included in any of the model presented below. Receiver linked to a HUB continuously received acoustic signals from both environmental and physiological sensors acoustically at frequency of 69kHz. The Hub was installed at a radius of ~200 m of the cage and was connected to WIFI of the farm to send data to the cloud and to display data in real time to live dashboard.

Growth performance and mortality data gathered over the trial were modelled using generalized linear models depending on environmental and physiological parameters recorded by different sensors. Selection of parameters to fit the best models has been carried out and only the best model for each variable is presented below.

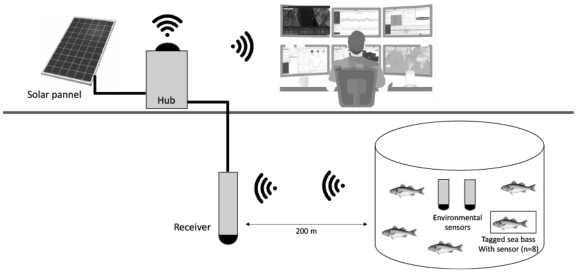

Fig. 1. Schematic of sensors deployment in the cage and connection of the sensors with the hub for transmitting environmental and fish physiological data farmer

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Results and discussion

Growth performance (i.e., weight gained by fish per day) was found to be significantly affected by both temperature and DO; the fish grew better with higher temperature and lower DO. It could appear surprising that fish grew better at lower DO but this can be explained first by the fact that the low DO values are overall associated to higher temperature occurring at the beginning of the trial (i.e. September), and so fish grew better. Also, even is the DO is overall low in this period (lowest measured at 40 % in some moments) and known to affect swimming and metabolism, this value is not significantly affecting critically the sea bass physiology. We also found a tendency for significant effects of both swimming activity and fish depth (p=0.054 and p=0.064 respectively). In more details, the growth performance tends to be enhanced when swimming activity recorded is greater, and fish is located near the water surface. This appears relevant because greater fish swimming activities are generally recorded when temperature is high. Overall, greater swimming activity is related to greater energy expenditure (Alfonso et al., 2021). However, greater energy consumed at higher temperature may be compensated by greater environmental conditions, overall favorizing better growth performances.

Also, we found that mortality is significantly affected by both temperature and the DO recorded. In more details, higher mortality is linked to low temperature and high DO levels. Oppositely to what has been observed for growth performance, the mortality did occur when the water temperature was cold. This is not very surprising but it is important to empathize that high temperature are also known to lead to such mortality events (Genin et al., 2020)kelp forests, and rocky shores. In this study, however, we found that the rate of onset (Ronset, but since we started the trial at the end of September, we did not face any huge warm vague triggering such mortality events, generally occurring during summer. In addition, the swimming activity and fish depth recorded by the physiological sensors implanted in fish were interestingly found to predict the mortality. High mortality over the experimental duration has been linked to greater swimming activity and fish located deeper in the cage. Therefore, here, contrary to what has been observed for growth performance, greater energy consumed could be linked to a response to acute stress event occurred in the sea cage which resulted in mortality events. Interestingly, location of fish at the bottom is known as thigmotaxis behaviour and indicative of stress in fish, including in sea bass (Sadoul et al., 2021), and could explain why mortality event are occurring in these moments.

In conclusion, predicting growth performance and mortality of sea bass using physiological and environment parameters of interest gathered by wireless sensors is very promising for the future, especially in the framework of precision livestock farming. It is, however, important to note that models and predictions have to be enhanced by including more parameters such as, species, salinity, turbidity, water current, stocking density, mass.

Acknowledgments

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BIOMONITORING PLANKTONIC THREATS IN SALMON AQUACULTURE - INSIGHTS FROM MICROSCOPY AND METABARCODING DATA

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Introduction

Salmon aquaculture loses c.20% of annual production as a result of gill disease triggered by plankton organisms, including jellyfish and harmful algal blooms - as such, health challenges from the plankton threaten the sustainability of the European salmonid aquaculture industry. Current methods of plankton monitoring lack sensitivity and specificity, can be time consuming, and require taxonomic expertise. For these reasons, data generated fail to provide actionable and timely information to aquaculture managers. We have previously provided proof-of-principle that environmental DNA (eDNA) metabarcoding may be deployed to identify and quantify multiple planktonic threats of salmon farms. In the current study we present a seven-month longitudinal planktonic survey of salmon aquaculture on the west coast of Scotland (UK), covering both spring and autumn blooms when the periods of highest mortalities in the farms typically occur. This dataset allowed us to assess biodiversity and abundance from DNA-based data in comparison with plankton morphological identification, and to identify planktonic threats for salmon health.

Materials & Methods

Metabarcoding, plankton morphology and fish health were readily combined to identify key drivers of salmon gill health. Two sea cage sites (Site A (exposed), Site B (sheltered)) were monitored for c. 200 days (March - October 2021) by daily eDNA, phyto- and zooplankton samplings. Prior to both morphological and molecular analyses, a hit list of species of interest was compiled based on previously published literature and personal communications with the farms in order to record target planktonic taxa causing salmon gill disease. This list would allow us to inform our industrial partners of our preliminary findings in case of a bloom, and to perform quality control on our species database for metabarcoding analyses. Using GLMs and morphological species data, we have so far been able to investigate potential effects of species abundances on the incidence of AGD and PGD scores and mortality. We first tested these relationships assuming no temporal lag between species dynamics and fish mortalities, as well as assuming that mortality lagged 1, 2 and 3 and 7 days behind species dynamics. Preliminary eDNA data indicate that the method is sensitive to detect both phyto- and zooplankton species of interest and that some species might have been misclassified via the microscopy method. Next steps using the full eDNA dataset will aim to benchmark the molecular method against the microscopy data and, combined with abiotic variables (e.g., temperature, salinity, turbidity), to assay their ability to predict salmon mortality and gill disease (AGD, PGD).

Preliminary results

Up to 96 phytoplankton and 74 zooplankton taxa were recorded after morphological identification analyses, including most of the target species from our hit list. The dynamics of the hit species as well as of the most abundant plankton groups are shown in Fig 1, where abundance of taxa is represented in a logarithmic scale. Regarding phytoplankton, the two sites presented similar species dynamics; the HAB genus *Pseudo-nitzschia* was present on both sites and was abundant from April onwards whereas a March *Skeletonema* bloom was only observed in site B. Regarding zooplankton, total abundances, as well as bivalves and ophiura larvae were higher in site B. The most abundant hydromedusae species from our target list were *Obelia* sp. and *Lizzia blondina*. The latter showed higher abundances during late summer, same as for doliolids (only for Site A) and *Oikopleura.dioica* (more clearly observed in Site B).

Preliminary data (Table 1) shows the potential of eDNA metabarcoding in detecting planktonic organisms at the species level when morphological analyses is only reaching to genus level. Moreover, our eDNA analyses will detect benthic life stages from meroplanktonic target species, allowing for a thorough analysis of the potential threats for salmon health that are in the water off the pens. We expect that our upcoming sequencing data will include many more taxa than those recorded by microscopy, hence improving our initial hit list. Lagged statistical modelling will enable the detection of species that have a significant immediate, delayed and cumulative impacts on fish health

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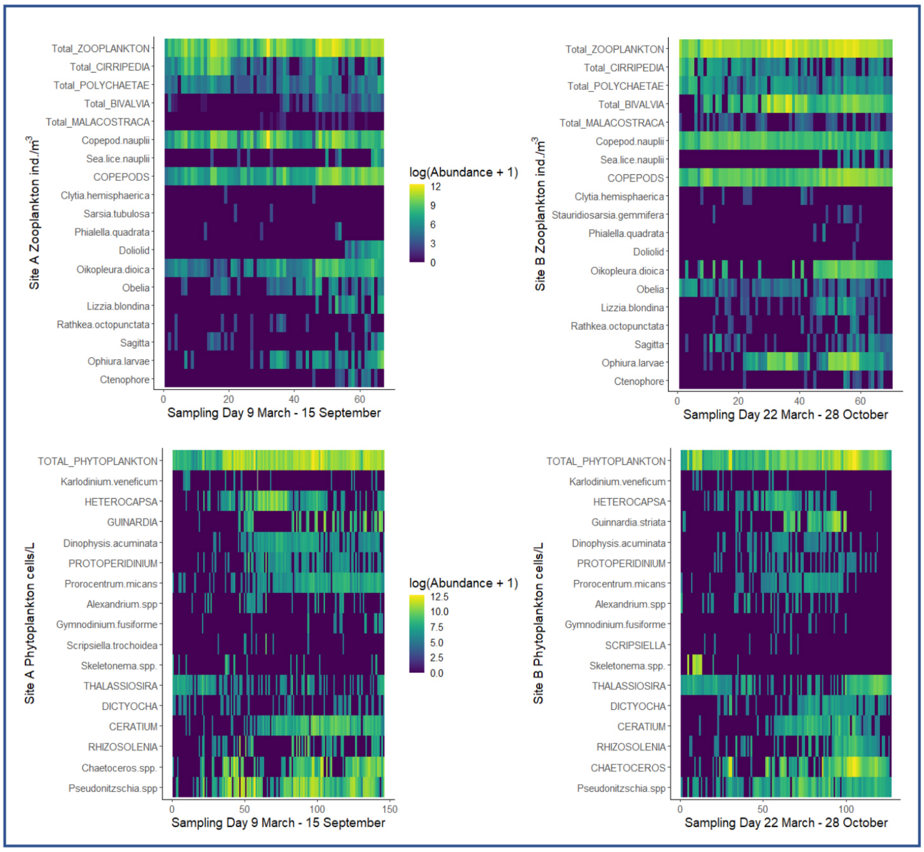

Fig. 1: Heatmaps representing zoo-and phytoplankton for Site A and B. Log scale.

Species	Microscopy	eDNA	eDNA sp	Species	Microscopy	eDNA	eDNA sp
ALEXANDRIUM	🟩			CERATIUM	🟩		
Aurelia aurita		🟦		Lizzia.blondina	🟩		
CERATIUM	🟩			Obelia	🟩	🟦	Obelia longissima / geniculata
CHAETOCEROS	🟩	🟦	Chaetoceros_socialis	Oikopleura.dioica	🟩		
Clytia_hemisphaerica	🟩	🟦		Ophiura.larvae	🟩		
Coscinodiscus_wailesii		🟦		Paramoeba		🟦	
Cylindrotheca_closterium		🟦		Phialella.quadrata	🟩		
DICTYOCHA	🟩			Prorocentrum_micans	🟩	🟦	
Dictyocha_speculum		🟦		PROTOPERIDINIUM	🟩		
DINOPHYSIS	🟩	🟦	Dinophysis_acuta	Pseudonitzschia.spp	🟩	🟦	Pseudo-nitzschia
Ditylum_brightwellii				Rathkea.octopunctata	🟩		
Doliolid	🟩			RHIZOSOLENIA	🟩	🟦	Rhizosolenia
Ectopleura	🟩			Sarsia.tubulosa	🟩		
Eucampia_zodiacus		🟦		SCRIPSIELLA	🟩		
Guinnardia.striata	🟩	🟦	Guillardia_theta	Sea.lice.nauplii	🟩		
Gymnodinium.fusiforme	🟩			Skeletonema.spp.	🟩	🟦	Skeletonema
Kareniaceae		🟦		Thalassiosira	🟩	🟦	Thalassiosira

Table 1. Detection of phytoplankton and zooplankton species of interest via microscopy and eDNA metabarcoding approaches.

Outcome

Our project will augment our proof-of-principle that environmental DNA monitoring can be used to detect planktonic threats (algae, jellyfish, amoebozoans) to salmonid aquaculture and potentially translate this into a low-cost on-site quantitative test for key drivers of Complex Gill Disease (CGD). Rapid and early identification of the planktonic threats will thus enable salmon farmers to take effective, timely steps to mitigate their impact on finfish. We hope our technology will provide a step change in mitigating losses caused by planktonic threats, improving both the sustainability and productivity of the salmonid aquaculture industry.

HYDROGEN SULPHIDE (H_2S) REGULATES THE MUCOSAL IMMUNE DEFENCES OF ATLANTIC SALMON (*Salmo salar*)

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Introduction

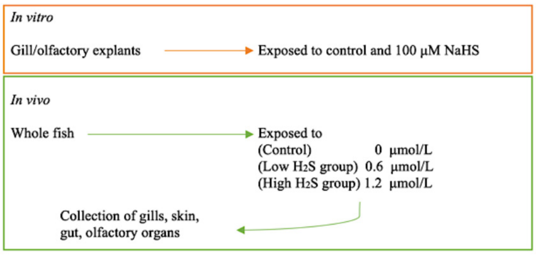
Fish are exposed to several chemical stressors and toxicants during their lifetime, which may cause damage and adverse effects on their physiological functions. Hydrogen sulphide (H_2S), a flammable gas characterised by the odour of "rotten eggs", is one of these chemical stressors. The gas is mainly produced by sulphate-reducing bacteria (SRB) by using sulphate (SO42-) as a terminal electron acceptor in the degradation of organic matter and directly reducing it to bisulphide (HS−) to produce H_2S under anaerobic conditions. H_2S has been a problem in land-based Atlantic salmon production particularly in recirculating aquaculture systems (RAS). Marine water contains more sulphates than freshwater, hence issues on H_2S toxicity are often encountered in saline production systems. High levels of H_2S may affect the fish health and robustness, and in worst case, can result to mass mortalities. Significant research studies have been done to explain its formation in the system, however, physiological and immunological studies designed to understand the mechanism of H_2S are scarce.

This problem is particularly relevant for mucosal organs that are exposed to this chemical stressor. Fish mucosal organs such as the gills, gut, skin, and the recently described immune organ of the nasal mucosa (olfactory organs), are considered the first line of defence against external threats and have a direct role in fish innate and adaptive immune responses. To further contribute to the understanding of the physiological impacts of H_2S in fish, the present study utilised mucosal tissues of Atlantic salmon.

Materials and methods

The study performed *in vitro* and *in vivo* exposure trials to investigate the biological functions of exogenously produced H_2S on the mucosal organs of Atlantic salmon. The *in vitro* trial explored the culture of gills and olfactory explants from Atlantic salmon smolts and exposed them to a sulphide donor, sodium hydrosulphide (NaHS). The *in vitro* trial further explored how mucus participated in H_2S-mucosa interaction and this was achieved by pharmacological treatment of the explants either to stimulate or inhibit mucus production, prior to NaHS exposure.

In another trial, Atlantic salmon smolts were transiently exposed to group of H_2S treatments for an hour followed by a 24 hr recovery period. The mucosal organs were collected for gene expression analysis and histology.



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Results and Discussion

The results from *in vitro* trial discovered the regulation of two important immune-related genes. The genes for sulphide-detoxification were found to be responsive in both mucosal explants which may likely suggest a stimulated toxicity reduction of H_2S and can be perceived as a protective mechanism of the mucosal organs from the toxic threats brought by H_2S. The sulphide donor NaHS also inhibited the expression of genes responsible for mucin secretion in olfactory organ explants suggesting a possible adverse effect to the integrity of the nasal mucosa and may likely provide less protection from the sulphide toxicity. The presence of a mucus stimulant causes differential modulation of mucin expression, which could potentially provide a sufficient protection in the olfactory mucosa from a subsequent dose of NaHS.

In vivo transient exposure trial showed that H_2S-induced stress provoked molecular and histo-structural changes in the four mucosal organs. Acute H_2S exposure influenced several immune-related genes encoding for mucins, cytokines, sulphide-detoxification enzymes, antioxidants, and apoptotic enzymes. While gut remained unaffected, gills and skin predominantly exhibited gene upregulation, whereas olfactory organ displayed a down-modulation. These distinct responses imply a diverse and probably a tissue-specific response exhibited by the mucosal organs as an adaptation and recovery strategy from H_2S exposure. The study also provided evidence that a synergy between pro- inflammatory cytokines and oxidative stress occurred in the induction of inflammatory response following an acute H_2S exposure, particularly in the olfactory organ and skin. In terms of histo-structural alterations, gills, gut and olfactory organs were moderately affected. Overall, H_2S possesses both stimulatory and inhibitory effects which interferes with Atlantic salmon mucosal immune responses.

Conclusion

The results reveal that exogenous source of H_2S regulates the mucosal organs of Atlantic salmon. Acute exposure to H_2S could alter both molecular and morphological immune defences of gills, skin, gut and olfactory organs.

Acknowledgements

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IMPROVING FEED EFFICIENCY IN FISH: LET'S GET DOWN TO BUSINESS

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A well-known challenge

European fish farmers have to face a competitive market where environmental concerns are now at the center of consumer choices. The feed distributed to fish is at core of sustainable development of aquaculture. On the one hand, feed is the first item of expenditure, and can represent up to 70% of the costs of producers (Besson et al., 2016)fish farming faces an increasing demand in fish products, but also various environmental challenges. Genetic improvement in growth rate and feed conversion ratio is known to be an efficient way to increase production and increase efficiency in fish farming. The environmental consequences of genetic improvement in growth rate and feed conversion ratio, however, are unknown. In this study, we investigated the environmental consequences of genetic improvement in growth rate and feed conversion ratio in an African catfish farm, using Recirculating Aquaculture System (RAS. On the other hand, feeds contribute to the exploitation of fishery resources and to degrading the environment around the farms, and are the main contributor to the global environmental impact of aquaculture in Life Cycle Analyses. The sustainable development of fish farming therefore depends on its ability to use less feed. One of the key levers to meet this objective is to improve the feed conversion ratio (FCR) of farmed fish through selective breeding (de Verdal et al., 2018). FCR represents the ability of fish to convert the feed distributed into fish biomass.

The killer question

Including feed efficiency as a trait in fish breeding programs, an accessible grail or a wonderful myth? We will review the latest results obtained on European sea bass (Besson et al., 2019, Rodde et al., 2021), gilthead seabream (Besson et al., 2022), rainbow trout, and Nile tilapia (de Verdal et al., 2022), in order to identify successes and remaining issues in the genetic improvement of feed efficiency, and to identify future directions.

The present strategies

During the past two decades, a huge effort has been deployed to tackle a simple but very hard problem: how to accurately measure feed intake of individual fish? Individual phenotypes are indeed the key for efficient genetic improvement of feed efficiency.

We will see how this question was either targeted (radio-opaque glass beads, isolated fish feed intake, chemical markers, video recording) or circumvented (screening of putative correlated indirect traits, including growth rates, fasting-tolerance, metabolic rate, behaviour) in different projects. Genetic variation, putative efficacy of pedigree and genomic-based selective breeding for feed efficiency through these strategies will be compared and discussed.

Which future opportunities ?

Prospects of improvement of feed efficiency with alternative methods to estimate feed intake including stable-isotopes strategies, or artificial intelligence-based feeding behaviour tracking (including fish recognition and pellets detection), will be exposed and discussed.

Acknowledgements

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SUBSTRATE CONSUMPTION PREFERENCE OF POTENTIAL BACTERIAL PATHOGENS ISOLATED FROM BIVALVES

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Introduction

Bivalves are widely distributed around the European coastline, at intertidal and subtidal zones (Bridge, 2012), being responsible for several ecosystem services (e.g., carbon storage and nutrient cycling) and representing an important nutritional and economic source for humans (Olivier et al., 2020). Due to its filtration characteristics, bivalves are highly exposed to pollution sources, concentrating contaminants from water, including potential pathogenic microorganisms that can cause several infectious diseases to humans (Olivier et al., 2020; Pereira et al., 2015). Therefore, since, traditionally, bivalves are consumed low or raw cooked, it may represent an important via of pathogen contamination to humans (Anacleto et al., 2014). Although depuration process is a useful method to eliminate or decrease the number of some microorganisms (e.g., *E. coli*), for others it may not be efficient (Martínez et al., 2009). Hence, there is the need of new efficient and rapid strategies to detect potential pathogenic microorganisms in bivalves. Thus, the main objective of our work is to characterize the substrate consumption preference of four potential pathogenic bacteria isolated from bivalves for human consumption.

Materials and methods

This study focused on the characterization of substrate consumption preference of 4 potential bacterial pathogens isolated from bivalves collected in Ria de Aveiro, Aveiro, Portugal. Namely, were tested the species *Pseudomonas aeruginosa* (*P. aeruginosa*), *Pseudomonas knachmussii* (*P. knachmussii*), *Vibrio pacinii* (*V. pacinii*), *Vibrio diabolicus* (*V. diabolicus*) and *Escherichia coli* (*E. coli*). The metabolic profile of each bacterium was analyzed using the BIOLOG® GEN III Microplates™ (Biolog, Hayward, CA, USA). Briefly, fresh grown bacteria were suspended in inoculum fluid with the recommended turbidity, followed by its inoculation in the microplates and incubation at 33ºC for 6 hours. The optical density (λ = 590 nm and 750 nm) was analyzed immediately (t= 0) and after each hour of incubation in the Biolog MicroStation device. The metabolic profile was estimated through the average well color development (AWCD) as described by Zhao et al. (2022).

Results and discussion

In Fig. 1 is represented the substrate consumption of each isolate tested during 6 h of incubation. It was possible to notice that *Pseudomonas*, has a high substrate consumption rate while *Vibrio* species seemed to take more time to consume the different substrates (Fig. 1 and Fig. 2). In fact, *Pseudomonas* genus is considered a ubiquitous microorganism mainly due to its metabolic versatility which may explain this result. Overall, among the substrates tested, the carboxylic acids, esters and fatty acids seem to be preferably consumed followed by sugars (Fig. 2). Nevertheless, worth to note that that each bacteria species seems to have a consumption pattern preference.

Concerning antibiotic tolerance, several antibiotic classes are incorporated in the BIOLOG plate GEN III. Hence, through the analysis it was observed that *P. aeruginosa* grow well in the presence of most of the antibiotics tested. On the other hand, *Vibrio* species showed lower growth rates.

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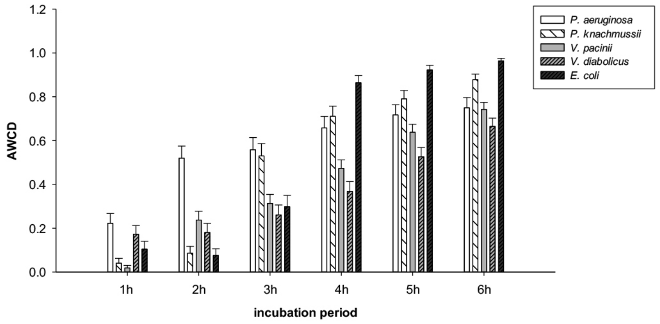

Fig. 1 – Substrate consumption estimated through average well color development (AWCD) for each bacterial strain tested: *Pseudomonas aeruginosa (P. aeruginosa), Pseudomonas knachmussii (P. knachmussii), Vibrio pacinii (V. pacinii), Vibrio diabolicus (V. diabolicus) and Escherichia coli (E. coli)*. Bars represent mean ± standard error.

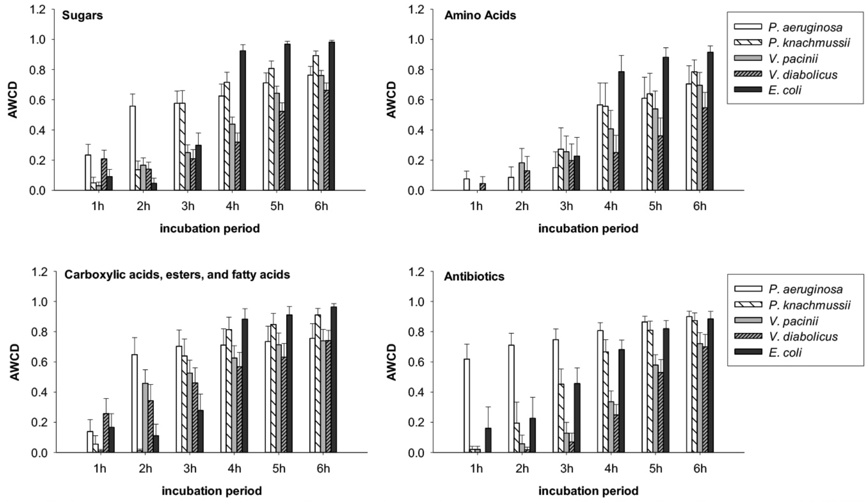

Fig. 2 – Substrate consumption estimated through average well color development (AWCD) for each type of substrate tested: sugars, amino acids, carboxylic acids, esters and fatty acids and antibiotics and for each bacterial strain tested: *Pseudomonas aeruginosa (P. aeruginosa), Pseudomonas knachmussii (P. knachmussii), Vibrio pacinii (V. pacinii), Vibrio diabolicus (V. diabolicus) and Escherichia coli (E. coli)*. Bars represent mean ± standard error.

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EFFECT OF A PLANT EXTRACT ON VIRULENCE GENE EXPRESSION OF A CILIATE RESPONSIBLE FOR OUTBREAKS IN TURBOT AQUACULTURE

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Background:

Infections in farmed fish are commonplace, often resulting in considerable fish production constraints. *Philasterides dicentrarchi* is a ciliate protozoa responsible for scuticociliatosis outbreaks in turbot aquaculture, and high mortality rates, resulting in significant economic losses (Iglesias *et al*., 2001)the causative agent is not always accurately identified. In this study, we identified two isolates of scuticociliates from an outbreak in cultured fine flounder Paralichthys adspersus. Scuticociliate identification was based on morphological data, examination of life stages and the use of molecular approaches. The isolates were compared with a strain of Philasterides dicentrachi from turbot Scophthalmus maximus and with a strain deposited in the American Type Culture Collection as Miamiensis avidus ATCC® 50180™ . The use of morphological, biological and molecular methods enabled us to identify the isolates from the fine flouder as P. dicentrarchi. Comparison of P. dicentrachi isolates and M. avidus revealed some differences in the buccal apparatus. Unlike P. dicentrarchi, M. avidus has a life cycle with three forms: Macrostomes (capable of feeding on P. dicentrarchi. Implementing safe and effective strategies to eliminate this organism has proven difficult so far (Shin *et al*., 2014), as pharmaceutical drugs effective against such infections have been shown to be detrimental to fish and the environment, putting fish and human health at risk (Dai *et al*., 2008; Ahilan *et al*., 2010). Comparatively to synthetic drugs, plant extracts have lower associated costs, are more biodegradable, induce lower environmental impact, and minimal side effects, making them an interesting alternative to explore (Ahilan *et al*., 2010; Shankar Murthy and Kiran, 2013)besides serving as a source of employment. Application of medicinal herbs in aquaculture is gaining success. Recently use of medicinal plant is considered as an alternative to antibiotics in fish health management The present investigation was designed to study the influence of two herbal additives viz., Phyllanthus niruri and Aloe vera on the growth and disease resistance in goldfish, Carassius auratus. The experiment was conducted with the adult fishes for a period of 60 days. After the 60 days of experiment, fish of each treatment were challenged by pathogenic Aeromonas hydrophila which was given by intraperitoneal injection and kept under observation for 10 days to record clinical signs and the daily mortality rates. Phyllanthus, at 1.5 per cent concentration yielded highest mean weight gain (1.769 g. There is little research on protozoan virulence factors, however, it is known that *P. dicentrarchi* secretes proteolytic enzymes to invade and disseminate within its host cells and tissues, representing one of its main pathogenicity characteristics (Paramá *et al*., 2004; Shin *et al*., 2014).

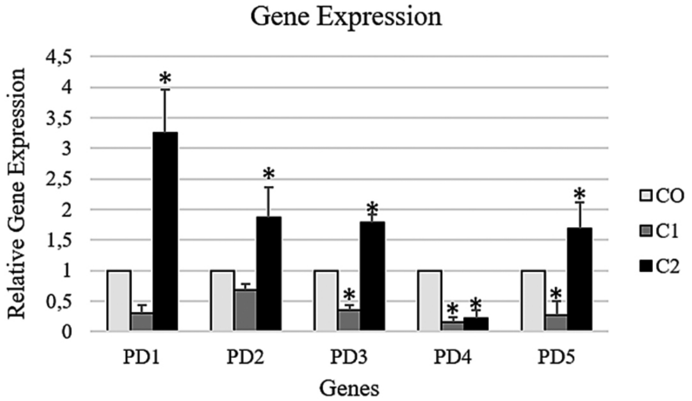

Figure 1 – Graphical representation of the effect of a plant extract on *P. dicentrarchi* at the expression level of virulence genes. Results were normalized using β-tubulin as a reference gene, and are expressed relatively to the negative control, which is thereby valued as 1. Bars represent relative gene expression ± standard deviation. PD1, PD2, PD3, PD4, PD5 represent the five assessed genes. CO – Control; C1 – 0.09 mg mL^{-1}; C2 – 0.83 mg mL^{-1}; * Significantly different from control ($p < 0.05$).

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Aim:

Evaluate the effect of a plant extract on the expression of virulence genes of *P. dicentrarchi*, to verify the potentiality towards the integration of plant-based strategies for disease control in aquaculture.

Methods:

The plant extract was obtained through the immersion of dried and grinded leaves in ethanol, incubation under orbital shaking, centrifugation, and solvent evaporation in a rotavapor to obtain the crude extract. *P. dicentrarchi* was exposed to two concentrations of the plant crude extract (C1 – 0.09 mg mL^{-1}; C2 – 0.83 mg mL^{-1}) plus the negative control (CO – 0 mg mL^{-1}) for 4h. The exposure period was then followed by RNA extraction, cDNA synthesis and RT-PCR (reverse-transcriptase PCR). Five relevant genes (PD1 to PD5) encoding proteases associated with *P. dicentrarchi* virulence were targeted for comparative analysis. Data was normalized with a reference gene, the β-tubulin gene, and expressed relatively to the negative control. Statistical analysis was performed via one-way ANOVA followed by the *post-hoc* multicomparison Dunnett's test ($\alpha = 0.05$).

Results and Discussion:

Exposure of *P. dicentrarchi* to a plant extract affected the expression of the five assessed genes (Figure 1). PD1 and PD4 expression was significantly increased at C2, however, PD3 and PD5 significantly decreased at C1 and increased at C2, while PD4 relative gene expression significantly decreased at both concentrations.

Our findings show that exposure to the plant extract induced a significant inhibitory effect on the expression of PD4 gene, although this trend had only occurred to the other genes under the lowest concentration of the plant extract. Nevertheless, these results evidence the potential feasibility of using this plant extract as a prevention strategy against *P. dicentrarchi* infections in aquaculture, representing an alternative to the application of antibiotics or other therapeutics, hence providing a safer option with lower environmental, economic, and health impacts on both farmed fish and the consumers (Voon et al., 2012).

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CONSUMER AWARENESS ABOUT AQUACULTURE IN EUROPE: A COMMUNICATION CAMPAIGN IN THE FUTUREEUAQUA PROJECT

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Introduction

Stakeholders believe that consumers play an important role in the promotion of aquaculture, especially regarding organic production (Lembo et al., 2018). Scholars have identified a general low awareness concerning aquaculture (Zander et al., 2018), which varies according to the species (Garza-Gil et al., 2016) and location (Froehlich et al, 2017). This low awareness persists despite media exposure (Papacek, 2018). However, consumers report some concern and awareness about general issues associated with seafood production, such as how it effects the environment (Jacobs et al., 2015a), its impact on the sustainability of fish populations (Bacher, 2016) and its influence on people's health (Jacobs et al., 2015b). Yet, there is lack of awareness about specific production methods, such as integrated multi-trophic aquaculture (IMTA) (Alexander et al., 2016) and labels associated with sustainable production in general (Feucht & Zander, 2015). The main objective of this study was to develop recommendations for social media communication strategies for increasing consumer awareness, perception and acceptance of European aquaculture. The recommendations were based on scientific literature, evaluations of the effectiveness of previous and current communication campaigns, the consumer survey results in previous stages of the FutureEUaqua project and experimental testing of the types of messages in social media that are preferred by consumers. The FutureEUAqua project focuses on social media as the preferred communication channel to influence consumers. Social media is considered to be the most appropriate channel to reach young consumers, who are a challenging group with falling seafood consumption levels.

Methods

After the evaluation of already available insights, a total sample of 2500 participants was recruited in the UK, France, Germany, Spain and Italy that were at least 50% responsible for the purchase and preparation of seafood in their household. They participated in an experimental survey and were randomly assigned to balanced groups that were exposed to one of five experimental social media posts that varied in tone (factual vs emotional) and visual representation (text vs text and image). Participants were then asked to evaluate the posts on comprehension, liking and willingness to repost. Participants also responded to explanatory topics, such as open-ended questions and consumer characteristics in terms of global innovativeness, attitudes towards nature, food technology neophobia, health concerns, as well as seafood purchasing and consumption behaviour. The last part of the survey focused on social and demographic characteristics, including region and country, coastal and inland location, occupation, education, household situation, gender, age and income.

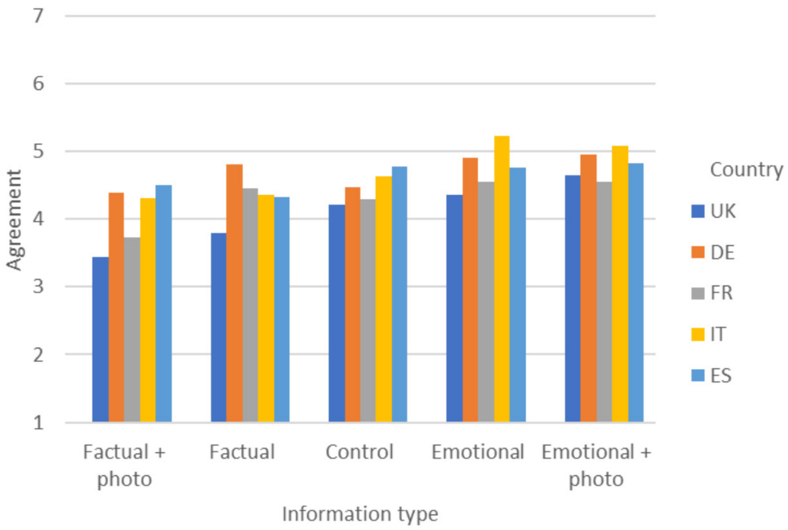

Figure 1 Country differences for liking of the five social media posts

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Results

The main results from the experiment showed a clear consumer preference for emotional content in social media posts (figure 1). Female participants seemed to report higher scores in general in this study. Age did not seem to have a major effect on the experiment. However, younger participants in the study seemed to like the control message less than other age groups, while they preferred the emotional message without a picture the most.

Conclusion

Based on the results, the FutureEUAqua communication strategy will use short, visually attractive and emotionally engaging social media posts to improve perceptions and increase awareness of and acceptance for aquaculture in Europe.

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ULTRAVIOLET B RADIATION EXPOSURE INDUCES TRANSCRIPTIONAL CHANGES IN GILTHEAD SEABREAM (*Sparus aurata*) REARED IN CAGES ON THE RED SEA COAST OF SAUDI ARABIA

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Introduction

Ultraviolet B (UVB) radiation has been recognized as a significant stressor for marine fish, particularly those confined to aquaculture cages. Gilthead seabream (*Sparus aurata*) has a high economic value, and it has been increasingly reared in offshore aquaculture systems in the oligotrophic and highly transparent Red Sea waters, which shows elevated underwater UVB levels. We recently reported that exposure to UVB daily doses from 3 to 12 kJ m^{-2} d^{-1} resulted in increased mortality, growth reduction, and typical sunburn, epidermal sloughing, and necrosis in the skin of *S. aurata*. Moreover, we also demonstrated that UVB induced changes in behavior, metabolism, physiology, immune system, and increased oxidative stress (Alves et al., 2020, 2021). With the aim to understand whether the responses and tissue damage previously reported in this species towards exposure to UVB are accompanied by overall transcriptional changes, the present study addressed the global gene expression profiles in the skin and immune organs after exposure to natural underwater UVB levels.

Material and methods

Juveniles (32.03 ± 4.17 g) were exposed for 43 days to two experimental groups: 1) UVB (daily dose, 6 kJ m^{-2}; representing levels between 5 and 7 m depth); 2) Unirradiated treatment, used as a control. Skin and immune organs (head kidney, spleen) were collected for RNA sequencing and for histopathology. Differentially gene expression analysis was performed in both skin and immune organs between control and UVB treatments. Lists of differentially expressed genes (DEGs) were subsequently used to perform gene ontology and pathways enrichment analyses. UVB-induced damage was also confirmed through histopathological examination.

Results

We described for the first time the transcriptional changes occurring in the skin and immune organs of *S. aurata* after long-term exposure to UVB. In the skin, 580 genes were up-regulated in the fish exposed to UVB, while 365 genes were down-regulated. In the immune organs, 34 genes were up-regulated in fish exposed to UVB, while 56 genes were down-regulated (Figure 1).

We identified several candidate genes in both tissues in response to UVB exposure, and several enriched GO terms and pathways were annotated in the skin after enrichment analysis. In the skin, enriched pathways in UVB-exposed fish were related to cell cycle, immune system and inflammatory response, proteasome, proteolysis, and oxidative stress response. UVB exposure up-regulated several key genes involved in cell cycle regulation, proteolysis, immune system and inflammation response, and antioxidant components. In contrast, UVB exposure inhibited the expression of several genes related to growth factor activity, cell growth and differentiation, and pigmentation (Figure 2).

We also demonstrated that UVB induced morphological changes in both tissues. Several UVB-induced lesions were identified in the skin, particularly an infiltration of immune-related cells resulted from an inflammatory response.

In the head kidney, we observed that UVB exposure induced a loss of tissue aggregation and impairment of intercellular integrity, accompanied by a down-regulation of several claudins and keratins genes, suggesting a disturbed tight junction function. UVB exposure also down-regulated genes related to angiogenesis.

This study provides noteworthy insights about the molecular changes in fish with long-term exposure to UVB. Our data can be used in the future to identify potential biomarkers and explore the mechanisms underlying the molecular changes occurring in fish reared in offshore aquaculture systems in oligotrophic and highly transparent waters.

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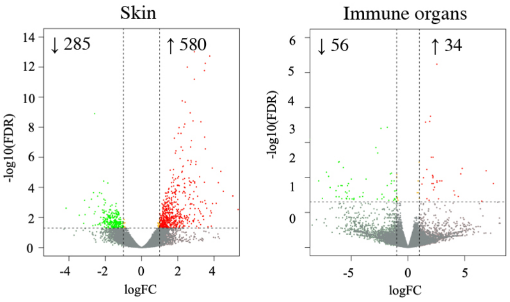

Figure 1. Volcano plot of DEGs identified in the skin and immune organs of *S. aurata* exposed to UVB. Each dot represents one gene. Red dots represent up-regulated genes (FDR < 0.05 and log2 fold change ≥ 1), and green dots represent down-regulated genes (FDR < 0.05 log2 fold change ≤ -1) in UVB-exposed fish.

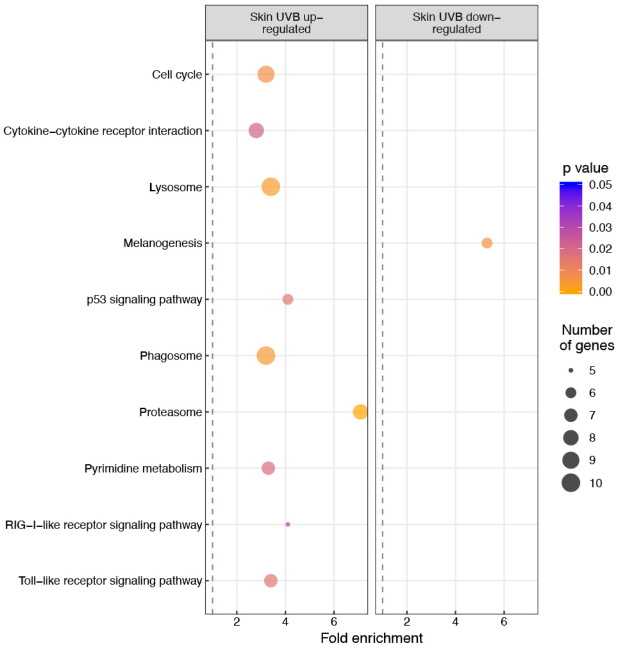

Figure 2. Dot plot showing the enriched KEGG pathways ($p < 0.05$) of DEGs (left, up-regulated; right, down-regulated) in *S. aurata* skin after UVB exposure. The vertical axis represents the enriched pathways, and the horizontal axis represents the rich factor of the enriched pathways. The size and color of dots represent the gene number and the range of p-values, respectively. Fold enrichment is the ratio of differentially expressed gene number enriched in the pathway to the total gene number in a particular pathway.

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EMBRYONIC DEVELOPMENT AND SENSITIVE STAGES OF ATLANTIC SALMON (*Salmo salar*) EGGS

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Introduction

Atlantic salmon (*Salmo salar*) aquaculture is of great importance to Norway, which is the global leader producer of this species (Ernst & Young AS, 2019). Egg and hatchery production is a crucial step in salmon aquaculture, as is it essential to provide good quality fishes throughout the whole production cycle (Blaxter, 1988). Although the Atlantic salmon is a well- established industry, little has been reported about early stages of development in relation to time, and sensitivity to mechanical stress (handling routines) during the embryonic period. Facing these research gaps, this study aimed to provide a detailed description of *Salmo salar* embryonic development in relation to time in degree days (dd) and to identify the sensitive stages from fertilization to hatching.

Materials and methods

Approximately 15000 eggs, pooled from three females, were fertilized with milt from one male and incubated at 8°C. From fertilization to hatching the embryonic development was recorded through pictures. To test sensitivity, a mechanical shock device that allowed to drop the eggs from predetermined heights, adapted from Jensen & Alderdice (1983), was used. Four different heights were used for dropping the eggs: 25 cm, 50 cm, 100 cm and 125 cm, and a control where the eggs were only handled as the others, but not dropped. The eggs were incubated for 7 days after they were mechanically stressed, and mortality was then recorded. LD10 and LD50 were estimated, which are the heights causing 10 and 50 % mortality, respectively.

Results

The embryonic development timing found in this study was, to some extent, similar to what was previously described by Gorodilov (1996). Discrepancies might be due to genetic variation and adaptation. This study provided a more practical embryonic development in relation to time in degree days, which is the main unit used by commercial hatcheries to follow development.

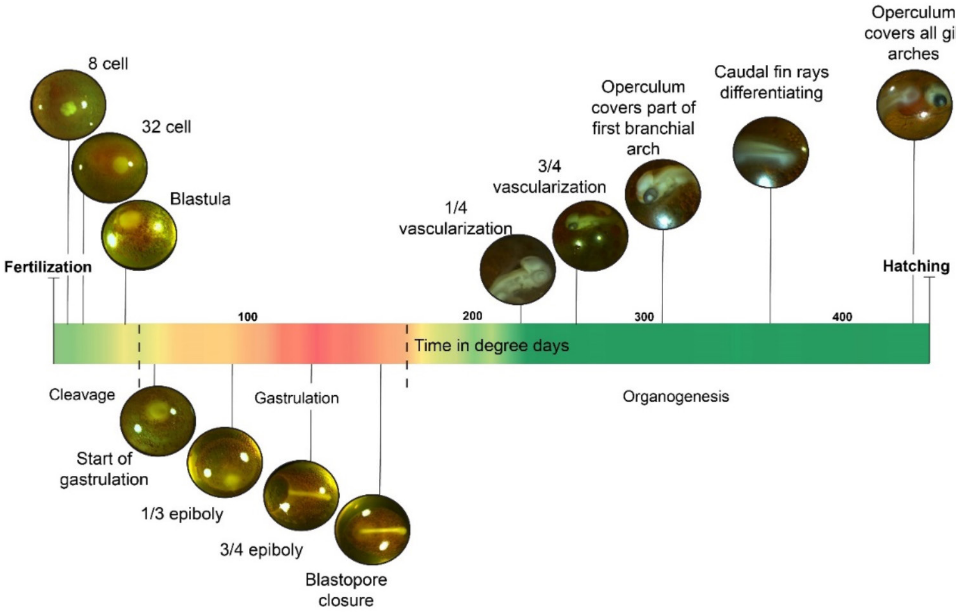

Figure 1. Timeline in degree days (dd) showing sensitivity to mechanical shock in the different stages and phases of embryonic development. Sensitivity is indicated by the color gradient. where green is less sensitive, and red is more sensitive. Different stages are demonstrated by the pictures, and the embryonic development phase are divided in cleavage, gastrulation, and organogenesis.

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Sensitivity observed during the embryonic development is illustrated on Figure 1. Embryos presented low sensitivity (LD50 > 100 cm) during the first 32 dd after fertilization. A steep increase in sensitivity was observed during gastrulation, where maximum sensitivity was reached, and the embryos were extremely sensitive (LD50 < 10 cm). Thereafter, at blastopore closure, sensitivity decreased, and it was very low until hatching. The results indicate that the window that the embryos were not sensitive to mechanical shock was wider than what was anticipated by commercial hatcheries. The study provided an overview of sensitive stages during the embryonic development of *Salmo salar*, but as the results in this experiment only accounted for mortality after 7 days from mechanical shock exposure, more studies are needed to determine other possible detrimental effects that do not result in mortality.

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EFFECT OF NOVEL DIETARY INGREDIENTS ON THE PHYSIOLOGICAL RESPONSES OF EUROPEAN SEABASS *Dicentrarchus labrax* SUBJECTED TO DIFFERENT REARING TEMPERATURES AND SALINITY OSCILLATION

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Introduction

Aquaculture is considered the fastest growing animal food-producing sector. If this expansion of aquaculture production is carried out with current practices it is destined to become unsustainable and contribute even further to the climate emergency, which is a growing challenge to the industry itself due to irregular weather patterns and abnormal environmental water parameters that may reduce fish performance, welfare and increase the risks of diseases outbreaks [1]. Aquaculture has relied on the incorporation of fish meal into aquafeed but due to ethical and environmental concerns and an inability to expand production [2], it is necessary that this ingredient be gradually phased out of aquafeed production, thus creating a need for novel ingredients. Multiple industries generate plant-based by-products with high amounts of indigestible carbohydrates and fiber, lower protein content and an unbalanced amino acid profile, which may limit their application in aquaculture feed. In a bid to improve these by-products, ingredients may be subjected to solid state fermentation (**SSF**), resulting in higher protein concentrations [3], higher bioavailability of nutrients [4] and bioactive compounds [5], thus providing better feed digestibility and higher bioavailability of bioactive compounds with health benefits that may increase animal resilience to stress caused by oscillating water conditions. This study aimed to evaluate the effects of dietary inclusion of fermented plant-based ingredients on physiological responses of European seabass subjected to different rearing temperatures under salinity oscillations.

Materials and Methods

Experimental diets consisted of one control diet with a 20% inclusion level of non-fermented plant-based ingredients mixture (Mix), and a test diet with 20% inclusion levels of same mixture fermented by *Aspergillus niger* in SSF conditions (Mix-SSF). Plant-based ingredients mixture consisted of soybean, rice bran, sunflower seed, and rapeseed meal (25% each). Both diets were isolipidic (18%) and isoproteic (42%).

In an environmental stress trial, seabass juveniles (22.4 ± 4.2g) were distributed into 24 experimental units. The trial occurred, simultaneously, in two recirculating aquaculture systems (RAS_1 and RAS_2). In $RAS_{1,}$ fish were kept in triplicate units at 3 temperatures (17°C, 21°C and 26°C) and fed the 2 experimental diets (control Mix and Mix fermented), in a 3 × 2 factorial design (total of 18 units). In Ras_2 fish were kept at a fixed temperature (21°C) and fed the two experimental diets (n= 3 repetitions/treatment, total of 6 tanks). During the six-week feeding trial, salinity was altered weekly in RAS1 whereas in RAS_2, salinity was kept constant (33 ppt). Growth performance, digestive enzymes, oxidative stress and innate immunity parameters were analysed at the end of the trial.

Results

An overall reduction in growth performance, regardless of environmental conditions, was observed in seabass fed the experimental fermented diet (Mix-SSF), which in part can be explained by a higher feed conversion ratio and a reduced feed intake due to an apparent reduction of dietary palatability.

Diet formulation did not have an effect on digestive enzymes activities. However rearing temperature and especially salinity oscillation significantly effected digestive enzyme activities. When comparing the two groups reared at 21ºC, fish exposed to different salinity conditions, showed a significant decrease in digestive enzymes activities when compared to fish kept at fixed salinity.

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Oxidative stress indicators were significantly affected by experimental diets and temperature and salinity conditions. Catalase and glutathione peroxidase activities showed an interactive effect, where fish reared at 21ºC showed higher enzymatic activity when fed the Mix-SSF. In contrast, fish reared at 17ºC and 26ºC showed higher enzymatic activity when fed the control diet (Mix). When comparing fish reared at 21ºC but exposed to different salinity conditions, fish subjected to salinity oscillation showed a reduction in redox status. Interestingly, the Mix-SSF diet reduced the effect of salinity stress.

Innate immunity parameters analysed were affected by rearing conditions with experimental diets having a significant effect on the alternative complement pathway (ACP). Fish fed the Mix-SSF diet showed higher ACP activity than those fed the Mix diet.

Conclusions

Despite the improvement of some antioxidant mechanisms and innate immunity parameters, the fermentation process of plant-based ingredients using *Aspergillus niger*, as carried out in this work, was unable to provide a more efficient ingredient for European seabass juveniles, leading to an overall decrease in growth performance.

Acknowledgements

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EFFECT OF SOLID-STATE FERMENTED PLANT-BASED INGREDIENTS IN DIET DIGESTIBILITY AND DIGESTIVE ENZYMES ACTIVITY IN EUROPEAN SEABASS *Dicentrarchus labrax* JUVENILES

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Introduction

For the last decades, the aquaculture industry has grown at a stable rate and currently, due to stagnant capture fisheries, is the sector responsible for seafood production growth [1]. Aquaculture has heavily relied on fish meal for feed production but this practice has raised sustainability concerns due to its environmental impact and an inability to expand production [2]. If aquaculture feed production continues with current practices, it will become unsustainable and potentially hinder aquiculture expansion. The supply of novel feed ingredients is essential to prevent aquafeed quality and availability from becoming limiting factors for the sector. Multiple industries generate plant-based by-products with high amounts of indigestible carbohydrates and fiber, lower protein content and an unbalanced amino acid profile, which may limit their application in feed production. These potential ingredients may be upgraded by biotechnological processes such as solid state fermentation (**SSF**), resulting in higher protein concentrations [3] and higher bioavailability of nutrients [4], thus potentially providing better feed digestibility.

This study assessed the *in vivo* digestibility of diets, including fermented plant-based ingredients, and the effect on digestive enzyme activity in European seabass, *Dicentrarchus labrax*, juveniles.

Materials and Methods

Experimental diets consisted of two control diets with 20% and 40% inclusion levels of non-fermented plant-based ingredients mixture (20Mix and 40Mix), and two test diets with 20% and 40% inclusion levels of same mixture fermented by *Aspergillus niger* in SSF conditions (20Mix-SSF and 40Mix-SSF). Plant-based ingredients mixture consisted of soybean, rice bran, sunflower seed, and rapeseed meal (25% each). All diets were isolipidic (18%) and isoproteic (42%).

The *in vivo* digestibility trial was performed in a RAS system consisting of twelve tanks with individual faeces sedimentation columns (Guelph system). Twelve homogenous groups of European seabass juveniles (initial body weight, 70.9 ± 0.3g) were hand-fed each experimental diet, in triplicate, *ad libitum* twice a day, for 6 days a week during the faeces collection period. At the end of the digestibility trial, fish intestine (with pyloric caeca) were sampled. Apparent digestion coefficients of experimental diets were calculated and digestive enzymes activity were analysed.

Results

A clear trend emeged regarding apparent digestion coefficients (ADC) of experimental diets, where an interactive effect between ingredient treatment and inclusion level of ingredients was observed. More precisely, solid-state fermentation of plant-based mixture improved ADC of dietary crude protein, energy and dry matter when plant-based ingredients mixture was included at a 20% level. In diets where plant-based ingredients mixture were included at a 40% level, solid-state fermentation of this mixture significantly reduced the ADCs of dietary crude protein, energy, and dry matter. ADC of crude lipids was not affected by plant-based ingredients mixture SSF and inclusion level of ingredients.

Regarding digestive enzymes activity, α-amylase activity was significantly higher in diets where plant-based ingredients mixture was included at a 40% level, regardless of the SSF of the mixture. Solid-state fermentation of plant-based ingredients led to significantly higher lipase activity, while the opposite was observed for trypsin activity.

Conclusion

This works shows that solid-state fermented plant-based ingredients are well digested by European seabass juveniles, increasing protein, dry matter and energy digestibility, as long as dietary supplementation was kept at a 20% level. This work also demonstrates the potential of this biotransformation process to contribute to the replacement of fish meal in the aquaculture industry.

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FORTIFICATION OF FARMED FISH WITH OMEGA-3 LONG-CHAIN POLYUNSATURATED FATTY ACIDS USING SUSTAINABLE AND COST-EFFECTIVE INGREDIENTS IN FEEDS

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Introduction

As consumers become increasingly aware of the importance of eating habits on their health and wellbeing, the demand for natural and functional foods is growing fast to meet the specific consumer requirements. Fish are generally regarded as a healthy food because they provide valuable nutrients as the omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), that have been unequivocally associated with a protective role against a number of human diseases (e.g. cardiovascular diseases, diabetes and obesity).

Driven by sustainability and economic issues, feeds for farmed fish suffered in the past decades a shift in diet formulation from fishery-derived resources (fish meal and fish oil (FO)) towards terrestrial agriculture ingredients. Such dietary modification has been promoting a marked reduction on omega-3 LC-PUFA contents of diets and farmed fish, which is undesirable from the consumer point of view. Hence, aquaculture industry needs to find new fortification strategies to tailor aquaculture products with such health promoting nutrients.

Omegapeixe project aims to bring to the market omega-3 LC-PUFA- fortified fish products produced with sustainable and cost-effective diets. For this purpose, new biofortified finishing feeds will be developed to maximize deposition of omega-3 LC-PUFAs on fillet of two important European aquaculture species, turbot (*Scophthalmus maximus*) and European sea bass (*Dicentrarchus labrax)*. Efficacy of dietary fortifications aiming to raise omega-3 LC-PUFAs in fish fillet will be dependent on the optimization of the relative proportion of dietary omega-3 LC-PUFA and careful selection of best sources to replace the unsustainable fishery-derived resources (e.g FO). In this context, the present study aimed to screen the fatty acid (FA) profile of different omega-3 rich ingredients available on the market and to assess their potential application in turbot and sea bass feed formulations.

Materials and methods

The FA profile of a high quality FO, the conventional source of omega-3 LC-PUFA in aquafeeds, and three commercially available LC-PUFA-enriched ingredients (salmon oil, a byproduct from the salmon industry; algae oil; and a blend of microalgae and macroalgae (Algablend)) was determined by Gas Chromatography(GC) with flame-ionization detection. Fatty acid methyl esters (FAME) contained in these ingredients were obtained through transesterification by acidic methylation. FAME were identified by comparison with known standard mixtures and quantified using the software GCsolution for GC systems. Each FAME was expressed as % of total FAME. The amount of FA expressed in % of dry matter (DM), were calculated using an internal standard (C23:0) as a reference.

Results

Among the four ingredients, FO exhibited the highest content of EPA (% tFA and %DM) (Table 1). On the other hand, the algae oil was characterized by high levels of DHA, resulting in higher EPA+DHA levels. Salmon oil and algablend) had the lowest concentration of DHA and EPA (% tFA and %DM), respectively.

Final remarks

The FA characterization revealed that algae oil due to its high content of omega-3 LC-PUFA, particularly DHA, is the most promising ingredient to be included in omega-3 LC-PUFA- fortified feeds. Although with a lower content of omega-3 LC-PUFA than algae oil or FO, the use of salmon oil in fortified diets is also appealing from an economic and a circular economy point of views. In vivo trials conducted with both species demonstrated that combination of these ingredients in feeds might be an effective fortification strategy to enhance omega-3 LC-PUFA content in turbot and sea bass fillets (please check the results in Monteiro et al., 2022 and Marques et al., 2022).

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Table 1. Omega 3 LC-PUFA profile (% total FA (tFA) and % DM) of the omega-3 LC-PUFA-enriched ingredients.

	FO	**Salmon oil**	**Algae oil**	**Algablend**
C20:5 n3, EPA % tFA	19.2	3.02	16.4	2.69
EPA % DM	14.7	2.11	11.9	0.47
C22:6 n3, DHA % tFA	9.64	3.29	38.1	29.6
DHA %DM	7.30	2.26	27.1	5.07
EPA+DHA %tFA	28.8	6.31	54.5	32.2
EPA+DHA %DM	22.0	4.38	39.0	5.54
DHA/EPA	0.50	1.09	2.32	11.0
Cost Ranking	**	*	***	****

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EFFECTS OF SIMULATED MARINE HEATWAVE ON THE ENERGY BUDGET OF A TROPICAL FISH SPECIES *Zebrasoma scopas*: INSIGHTS FOR OPTIMAL PRODUCTION IN ORNAMENTAL FISH FARMING

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Introduction

Marine heatwaves (MHWs) are extreme events featuring abnormally high water temperature for a prolongued period (from days to months) (Holbrook et al., 2019). This phenomen is expected to increase in frequency, duration and severity over the century (IPCC, 2021). MHW can have particularly significant impacts on marine habitats and ecosystems, deeply affecting organisms' physiology, namely their metabolism, growth and feed intake, thus compromising their energy budget (i.e. the energy intake and expenditure within the whole organism) and, consequently, their fitness and survival rates (Jobling, 1994). Even though the effects of ocean warming on the energy budget (EB) of marine organisms are beginning to be understood (e.g., Anacleto et al., 2018), the impacts of short-term exposure to high temperatures on fish' physiology have yet to be investigated under a MHW context. Therefore, the aim of the present study was to assess the effects of a simulated MHW (category IV; ΔT=+2 °C, 11 days) on the energy budget (i.e. growth, excretion, metabolism and food consumption) of juvenile surgeonfish (Zebrasoma scopas), a tropical species particularly popular in the marine ornamental fish trade, whose stocks largely rely on wild populations and are, thus, particularly susceptible to such events. In fact, the specimens' collection site (Maldives) has been exposed to repeated and severe MHWs during the last decades (1998 and 2016), with massive ecological and socioeconomic costs.

Materials and methods

Juvenile surgeonfish specimens (n=48; 4.7±1.4 g total weight, 5.8±0.6 cm total length) were collected in Maldives in February 2020 and brought to Guia Marine Laboratory (MARE-FCUL, Cascais, Portugal) from Tropical Marine Centre (TMC, Lisboa). Fish were distributed and maintained in 8 tanks (2 treatments x 4 replicate tanks) with independent recirculation aquaculture systems. After an acclimation period at control temperature conditions (29 °C), fish were exposed to two treatments: i) control scenario (29 ºC) for 11 days (n=16, i.e. n=4 per tank); and ii) simulated scenario of a category IV MHW (31 °C) for 11 days, followed by a 10-days recovery period at 29 ºC (n=32, i.e. n=8 per tank). In the MHW treatment, the water temperature was increased around 0.5 °C per day. A 30 years dataset for seawater surface temperature in the region of Maldives was acquired from NOAA (Daily Optimum Interpolation SST version 2; Banzon et al., 2016). The heatwaveR package (Schlegel and Smit, 2018) applies the MHWs definition by Hobday et al. (2018) and was used to determine the average duration (11 days) and the maximum temperature registered (31.3 °C; corresponding to a category IV MHW). At the beginning and after the exposure period, two specimens per replicate/tank were randomly weighted and used to determine EB parameters [growth-G, excretion (faecal-F and nitrogenous losses-U) and metabolism-R] in order to calculate the food consumption-C (C=G+F+U+R). To calculate G, fish were weighted frequently (2-3 times per week) and F, faeces were collected daily, oven-dried at 65 ºC to constant weight and stored at -20 °C. Then, the energy content was measured in fish and faeces by combustion in an adiabatic calorimeter pump. The nitrogenous compounds (ammonia - U) were determined according to Anacleto et al. (2018). Metabolism (R) was calculated as: 100 – G - F - U. Biochemical analyses (dry matter, ash, total lipids, crude protein and gross energy) were determined in the diet and fish body according to the AOAC (2005).

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Results and Conclusions

The energy proportion channeled for growth (49.2%) dominated the mode of energy allocation of juvenile *Z. scopas*, followed by excretion via faeces (39.5%). However, this trend was reversed under MHW exposure. It was also found that such exposure led to significant higher protein content in whole fish body and that higher percentage was spent for U in comparison to the control treatment. Moreover, under the recovery MHW period, higher feed intake (FI), feed efficiency (FE) and relative growth rate (RGR) were observed compared to both MHW and control treatments. At the beginning of the experiment (day 0), significantly higher viscerosomatic index (VSI) was found in comparison with MHW and control treatments determined at day 11; higher VSI was also detected under MHW recovery period than after MHW exposure. This study clearly demonstrated that the increased intensity and frequency of such extreme events will affect these tropical fish species, which may be further exacerbated by cascading effects throughout their ecosystem. Additionally, since surgeonfish species is vulnerable to MHWs and has been suggested as a potential candidate for ornamental fish farming (e.g., Holcombe et al., 2022), the data obtained are essential to understand how this species uses the resources ingested, and thus, to optimize its diet in aquaculture to avoid the capture of wild animals. Moreover, since MHW will occur in combination with other environmental drives of change (e.g. acidification, hypoxia), testing multistressor-related climate change scenarios are greatly needed to better understand future ecological impacts, and also for the development and implementation of management and conservation strategies in coral reef ecosystems.

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CO-CULTIVATION OF RAINBOW TROUT (*Oncorhynchus mykiss*) AND SEA LETTUCE (*Ulva fenestrata*) IN A RECIRCULATING AQUACULTURE SYSTEM

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Introduction

One of the cultivation strategies in marine aquaculture is integrated multi-trophic aquaculture (IMTA), where several species from different trophic levels are co-cultivated in the same, or connecting, water systems. Nutrients from the fed species are consumed by extractive organisms. This leads to an increased proportion of the initially added nutrition being utilized for biomass production, which improves the ecological sustainability of the aquaculture system [1]. Previous co-farming experiments have also found positive effects in addition to those emanating from the available nutrients, including improved growth and quality of the extractive species as well as improved fish survival, growth, and immune responses [2]. The aim of this study was to evaluate the effects of co-farming rainbow trout (*Oncorhynchus mykiss*) and sea lettuce (*Ulva fernestrata*) in a recirculating aquaculture system (RAS) on water quality. Health and welfare of the rainbow trout was evaluated through non-invasive welfare indicators, plasma cortisol levels and primary barrier function of skin and intestine. The performance of the Ulva was evaluated as change in biomass growth, nitrogen content and protein content.

Methods

In this experiment, 363 rainbow trout of 115 g ± 2.46 (mean ± SEM) amounting to a total of 41 kilos were housed in 6 tanks (400 L) in a RAS system (total volume 15 m^3). The co-cultivation trial lasted for 16 weeks and was divided in four periods of four weeks each. During the first and third period, only rainbow trout was held in the system. During the second and fourth period 1200 and 1000 grams of Ulva respectively, were cultivated in plastic bags (200 L) in a separate section of the same RAS system (Figure 1).

Water samples were taken from the inflow and outflow of the Ulva bags three times per week throughout the experiment and analysed for pH, NO_2^{2-}-N, NO_3^--N and NH_4^+-N. Ulva was sampled for total biomass once per week and 10 randomly selected Ulva individuals were frozen and later analysed for nitrogen and protein content. Fish welfare was evaluated through scoring of five non-invasive welfare indicators by visual observation every second day, as well as through final sampling of 12 individuals at the end of each four-week period. At sampling, fish were randomly netted, euthanized in 12 mg/L of metomidate and killed by a blow to the head. Blood was sampled, centrifuged and plasma stored at -20 C for later analyses of cortisol using RIA. Live skin and two intestinal regions were sampled for direct analyses of barrier functions using the Ussing chamber technology.

Results and Discussion

The results suggests that it is beneficial to co-cultivate Ulva with rainbow trout. The Ulva biomass increased during the co-cultivation periods, and nitrogen content as well as protein content increased from the start and end of the initial co-cultivation period. Regarding fish health and welfare no effects were found on the visually scored fish welfare indicators or plasma cortisol levels. Intestinal health parameters were slightly affected by the co-cultivation with Ulva with increased active transporting- but decreased barrier functions as a result.

Water quality parameters of dissolved inorganic nitrogens indicated no differences in net system concentrations in response to addition of Ulva. There was an increase in water pH after passage through the Ulva holding bags, indicating that Ulva positively affected the pH locally. This however, did not results in any overall net effect on the entire system. These results taken together show that the Ulva biomass used in the current experiment had the ability to affect the water quality locally, but not overall in the entire system, suggesting that the Ulva biomass was too low in relation to fish biomass and system water volume. Future studies will include adjustment of the relative biomasses in order to more thoroughly investigate possible effects on fish health and welfare.

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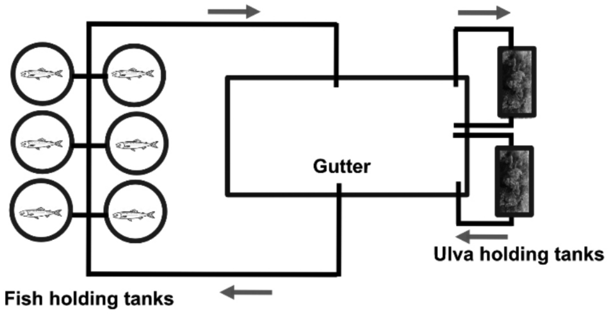

Figure 1. Schematics of the experimental recirculating aquaculture system (RAS). Grey arrows indicate water flow direction.

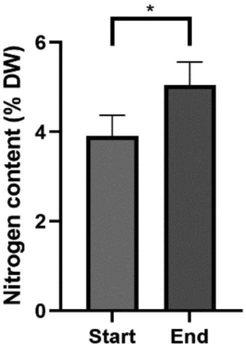

*Figure 2. Nitrogen content of Ulva at the start and end of the first co-cultivation period. Statistical significance is presented as * $p < 0.05$.*

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CREATING NEW LIFE FOR DISCARDED FISHING AND AQUACULTURE GEARS: PREVENTION OF MARINE LITTER GENERATION BY CONTRIBUTION TO MORE CIRCULAR AQUACULTURE ROPES

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Introduction

The new Circular Economy Action Plan adopted by the European Commission is driving a change, pushing up the circularity of the existing materials in aquaculture and fishing gears. When aquaculture or fishing gears become worn out, the end of life (EoL) treatment must be considered, but unfortunately, current EoL possibilities are not consistent with the waste hierarchy. Rather, fishing gear waste is often dispensed in the ocean. Abandoned, Lost, or Otherwise Discarded aquaculture and fishing gears (ALDAG and ALDFG) causes not only economic losses and hazards to safety at-sea to aquaculture farmers and fishers, but also impact on marine fauna and can pose substantial ecological and socioeconomic problems. Aquaculture and fishing sectors are associated with 14% to 38% of the items recorded in the surveyed beaches in the north-west of Spain, while they may represent the 54% in weight of floating marine litter in the south-east of coastal waters of the Bay of Biscay. Precisely, in the south-east coast of the Bay of Biscay, the cost caused by a kilogram of marine litter ranges between 0.3 to 6.7 EUR/kg.

In line with the new Circular Economy action Plan, this study aimed at demonstrating the feasibility of using an alternative material for gear making. To do so, discarded fishing nets were recovered, conditioned and recycled into ropes for mussel aquaculture in longline systems; hence, closing the loop, “from the sea for the sea”. The bottlenecks of the process undertaken to converting discarded nets into aquaculture ropes were identified. Furthermore, the performance of the ropes as on-growing ropes were tested at sea in a commercial aquaculture site, where mussels growth (size, weight and condition index), and ropes’ performance (breaking point) were monitored in a production cycle of one year. Technical, economic and environmental assessments were carried out to compare prototypes manufactured with recycled nets with conventional ropes. This study demonstrated that polyamide and polyolefin fishing nets can be recycled and converted in longline ropes for mussel aquaculture.

Material and Methods

Discarded EoL fishing nets and aquaculture ropes were samples from Basque fishing ports. Once the EoL material was recovered and classified, four materials were preselected for upcycling by mechanical recycling (polyolefin fishing nets, polyamide fishing nets, on-growing mussel ropes and holding ropes used to attach on-growing ropes to long lines). Finally based on performance and stock, two waste streams were selected to undertake the whole recycling process: Polyamide-based purse seine nets (PA) and polyolefin-based trawler nets (PO). The recovered nets were conditioned and mechanically recycled into recycled pellets (rPA and rPO pellets) and then monofilament were produced. With the monofilament aquaculture ropes were manufactured. Recycled and conventional ropes (as control) were tested at sea in an industrial longline system for one mussel production cycle. The sea test served to estimate the potential productivity of the ropes and their lifespan, by assessing; growth and survival of the mussels on the ropes, the condition index, the meat yield, the proximal composition and fatty acid profile of the mussels, the concentration of microplastics within the mussels, and physical properties of the ropes (tensile tests and durability traits). From the whole process, 22 technical indicators were calculated for each prototype as for the commercial rope (the reference system). The economic assessment was carried out through the material flow and the cost benefit analysis along the whole value chain of the aquaculture ropes, scaling from the laboratory level to industrial level. The economic flow between different productive sectors was analysed through the Input-Output methodology. Four economic indicators were calculated for each prototype rope. Life Cycle Assessment (LCA) methodology, according to ISO standards 14040:2006, was carried out to identify, quantify and compare environmental impacts linked to the ropes. Sixteen environmental indicators were assessed for each prototype of rope. Environmental social and economic assessment were carried under the umbrella of the circular economy

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Results

As a general outcome of the test at sea, it can be concluded that both recycled prototypes, rPA and rPO, presented similar results as the commercial one in terms of production yield, product quality, durability and functionality at sea and the occurrence of microplastics in mussel. Therefore, it can be stated that from the technical point of view, recycled ropes, especially the polyamide-based ropes, can be a suitable alternative as on-growing ropes for mussel aquaculture, thus, helping aquaculture industry to achieve the recycled targets proposed by the Circular Economy strategies.

From the environmental perspective, the life cycle indicators stated that the rPO ropes are better options than rPA or conventional ropes. From the economic perspective and at industrial level, recycled prototypes were more costly than commercial rope (75% and 59% more the rPA or rPO, respectively). Thus, to implement the recycled ropes in the market is required a price premium of, at least, 2-4% over the final aquaculture product (mussel) to compensate the higher costs of the recycled ropes. Despite the success of the technical characteristics of the recycled ropes, rope manufacturers still show little interest to exploit them in the short-term, unless the price premium would be covered by incentives, market demands, or a mandatory measure that would exist to promote recycling EoL gears as secondary raw material for new gear manufacturing.

MARINEHOLMEN RASLAB, PROVIDING DIVERSE AN INNOVATIVE OPTIONS FOR CONDUCTING SEALICE *Lepeophtheirus salmonis* RESEARCH

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Introduction

Marineholmen RASLab is a research and innovation company with a focus on in Recirculating Aquaculture System technology. Located in Bergen, at the heart of the aquaculture industry in Norway, RASLab offers state-of-the-art facilities for research into sustainable land-based fish farming. By re-using water in a closed loop RAS system, fish can be grown intensively, using less water resources, and producing a virtually zero environmental impact with a smaller footprint. The technology for providing the life support systems, cleaning the water, and supplying the fish with clean oxygenated water allows us to use technology to advance and transform the future of fish farming. Marineholmen RASLab has 12 self-contained RAS systems, that can run with fresh-, brackish-, or saltwater and are fitted with the latest technology allowing us full control of each system. Providing the capability to control feeding, light regimen, salinity, pH, temperature, and flow in all systems. This provides the opportunity to have replicate systems when conducting feed and treatment trials.

What we offer

Our RAS setup allows us to conduct research in:

- Water quality management
- Feed research
- Bioreactors
- Microbiology
- Sludge and waste treatment
- Sensor development.

We offer general project development and planning, which is vital when working with product development. Working to GLP standards, we ensure high quality results and data handling. This has allowed us to conduct a range of pharmaceutical efficacy and residue contracts in the sealice, *Lepeophtheirus salmonis*, which is a problem parasite affecting Atlantic salmon in the northern hemisphere.

Sealice, *Lepeophtheirus salmonis*, research at Marineholmen RASLab

Sealice are a threat to farmed Atlantic salmon, resulting in huge losses to the industry due to increased handling costs, reduced fish health, and decreased product quality. Methods of treatment and control have been developed to reduce the number of parasites in the salmon farms, however, resistance development has resulted in reduced treatment efficacy. This means that the industry needs to continue identifying and developing new treatment strategies.

Recently we have expanded services offered and can conduct *in vitro* and *in vivo* sealice (*Lepeophtheirus salmonis*) trials. Marineholmen RASLab has the capability to conduct both small scale *in vitro* pilot trials to determine the efficacy of newly identified treatments to larger scale *in vivo* dosage trials. The ability to control different water parameters allows us to conduct a series of trials over time while still being able to compare the results.

By controlling the water temperature, we can control the sealice growth rate, for example fish infected by copepodids at 12°C will reach the preadult II stage in 12-14 days. Decreasing water temperature will slow the growth rate, while increasing the temperature will increase the growth rate. We can therefore simulate real world conditions to determine whether treatment efficacy is affected by seasons. Or we can conduct treatments targeting specific life stages, which can be difficult when working at ambient temperature.

Conclusion

Marineholmen RASLab can provide facilities and knowledgeable staff to conduct product development research in the aquaculture field. Working to GLP standards we provide project planning and management which is vital for laying the groundwork for testing new products. We believe that strong and reliable research will aid in the improvement and development of the aquaculture industry.

ASSESSING THE POTENTIAL OF AN I-RAS SYSTEM USING *Ulva* sp. AND *Sparus aurata*

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Introduction

Recirculating Aquaculture Systems (RAS) is a technology well known for reducing water waste and leading aquaculture towards a more sustainable path. Integrated Multitrophic Aquaculture (IMTA) is another key system for a sustainable aquaculture by integration of low trophic organisms, such as seaweeds for high efficiency removal of nutrients in fed aquaculture. The combination of RAS and IMTA, I-RAS, aims to achieve maximum nutrients' reutilization and the creation of a new valuable product, increasing economic return and improving animal welfare (Qui *et al*., 2022). Gilthead seabream (*Sparus aurata*), is a species with a major aquaculture interest, largely cultivated in RAS systems and off-shore cages on the Mediterranean and with special economic interest in Europe (FAO, 2022). Ulva, a green seaweed, is a promising candidate for an integrated system, since it can assimilate dissolved inorganic nutrients (DIN) at a fast rate, use it to build up its biomass.

The aim of this study is to test and optimize the operation of a I-RAS system with Gilthead seabream and Ulva.

Material and Methods

Each of two identical I-RAS (Figure 1) systems were designed so that water from fish and seaweed cultivation tanks is collected in the main filter before re-distribution. Oxygen is added to the main filter. Gilthead seabream originated from EPPO-IPMA broodstock, reared and maintained in the facilities until the experiment started. Ulva was collected at EPPO, cleaned and kept for 3 weeks in a tank with seawater (SW) and aeration, until the start of the experiments. Non-mature bioballs were mixed with matured bioballs for 1 month, and then transferred to the I-RAS system for 9 weeks before this experiment. The experiment was 6 week-long, starting in 15/06/2022. Initial fish density was 11 kg m^3 with a mean 265,28 ± 51,60 g fish weight, one I-RAS was tested without seaweed and one with ulva, at 1, 2 and 3 kg m^{-2}, in triplicate.

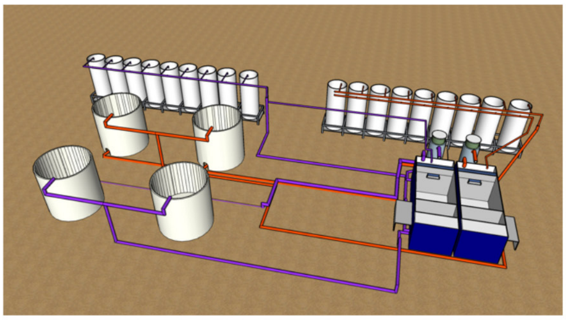

Figure 1. Overview design of the I-RAS systems. Each color represents the piping of one I-RAS system, comprising two fish tanks of 3000 L, nine seaweed tanks of 200 L, one protein skimmer, one 1650 L main filter (with mechanical and bacterial filtration), and one refrigeration unit.

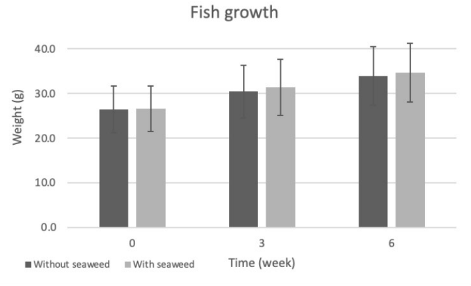

Figure 2. Growth of *Sparus aurata* on a I-RAS system during the 6 week-long trial.

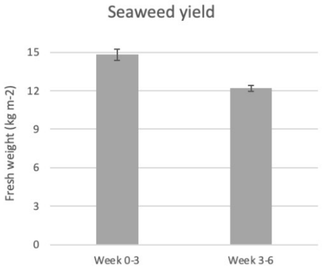

Figure 3. Ulva 3 week cumulative yield on the I-RAS system.

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Temperature and oxygen were measured twice a day, pH every day in the morning and salinity was kept at 36. Water samples were taken regularly, filtered, and frozen for posterior nutrient analysis. Feed was given for 1.4% of body weight according to the manufacturer's recommendation and adjusted weekly. Evaluation of fish growth took place at week 0, 3 and 6 were the weight, standard and total length were measured. To assess macroalgae growth, total fresh weight (FW) was measured, by removing the excess of water and weighing once a week. Ulva excess biomass was removed weekly and cultivation restarted at original stocking densities.

Results

During the experiment, mean water temperature was 23,59 ± 2,4°C, oxygen was 4,94 ± 1,01 mg L^{-1} and pH was 7,03 ± 0,19, for all fish tanks. Fish growth is presented on Figure 2, condition factor (K) for the system with seaweed and without seaweed were 1,72 ± 0,003 and 1,67 ± 0,10 initial and 1,79 ± 0,020 and 1,72 ± 0,064 final, respectively. Survival rate was 99,6 ± 0,46 in all fish tanks.

On all seaweed tanks, water temperature was 24,21 ± 2,49°C, oxygen was 7,6 ± 1,05 mg L^{-1} and pH was 7,65 ± 0,29. Ulva yield (FW) is presented on Figure 3.

BIOCHEMICAL COMPOSITION OF *Hediste diversicolor* REARED ON AQUACULTURE SLUDGE AND ITS POTENTIAL AS AN AQUAFEED RESOURCE

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Introduction

Sustainable growth of the salmon aquaculture industry is highly dependent on the availability of new feed resources as well as improved management and utilization of waste streams from production (Waite et al., 2014, Tacon et al., 2011). Polychaetes are capable of recapturing and recycling valuable biomolecules contained in aquaculture sludge and at the same time constitute a possible resource for aquafeeds (Wang et al., 2019b). In this study, the potential of *Hediste diversicolor* (OF Müller, 1776) to recover nutrients and energy contained in two types of salmon sludge was evaluated.

Material and methods

A 30-day feeding experiment was conducted assess the effect of two different diets, smolt (S) and post-smolt (PS) sludge, and different feed levels (A-D) on polychaete growth and biochemical composition. Feed levels were calculated based on estimated nitrogen content of diets and polychaetes; the different treatment groups (SA-SD, PSA-PSD) received feed equivalent to 5-40% of the nitrogen contained in polychaetes per day. Growth was calculated following Jørgensen (1990). Sample composition of polychaetes and sludge was analyzed with regards to ash content, total lipid (Bligh and Dyer, 1959), fatty acid profile (Metcalfe et al., 1966), protein, total amino acids and composition (Šližytė et al., 2017).

Results and discussion

Survival ranged from 80-90%, independent of treatment. Specific growth rates in the highest feed levels were significantly higher than in the lowest feed levels and growth was not affected by the type of diet. Similar to other studies, total lipid and total fatty acid contents of polychaetes were positively correlated with feed level while the type of diet had a limited effect (Wang et al., 2019a, Wang et al., 2019b). Fatty acid composition in polychaetes reflected feed levels: in both diet groups, lower feed levels showed a higher percentage of saturated fatty acids, monounsaturated fatty acids increased with increased feed supply in the smolt group and polychaetes fed with post-smolt sludge showed increased polyunsaturated fatty acids concentrations, specifically of DHA, with increasing feed levels. Neither diet nor feed levels affected protein content and amino acid composition in the polychaetes.

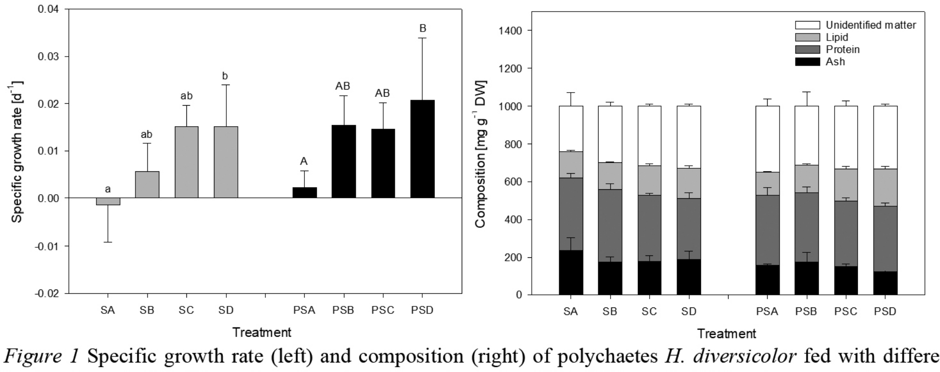

Figure 1 Specific growth rate (left) and composition (right) of polychaetes *H. diversicolor* fed with different levels of smolt (SA-SD, n=4) and post-smolt waste sludge (PSA-PSD, n=4). Different superscripts indicate significant differences ($P < 0.05$).

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Conclusion

Overall, *H. diversicolor* cultivated on both smolt and post-smolt salmon sludge was rich in proteins and lipids as well as essential amino and fatty acids, and contained suitable levels of trace elements that match the salmon industry's requirements. Therefore, the species can be considered to not only be valuable for recycling nutrients from salmon aquaculture side streams, but also serve as a potential resource to replace high-value marine ingredients in aquafeeds.

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AUTOMATIC DETECTION OF GROWTH-STUNTED PHENOTYPE IN FARMED ATLANTIC SALMON: A NEW INSIGHT INTO QUANTIFY THEIR DISTRIBUTION AND BEHAVIOUR BASED ON A MACHINE LEARNING APPROACH

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Introduction

In Norwegian salmon farming, about 15% of all salmon are lost during the production phase at the sea. Large sea cages which can hold up to 200,000 individuals are commonly used in Norway, but we have no established technology to properly monitor the fish. Some individual fish may present low growth, emaciated body conditions, and aberrant behaviour (Folkedal et al., 2016), which are referred to as "growth-stunted" (GS) individuals ('loser fish'). Their presence may be an indication of an inappropriate farming strategy (Noble et al., 2018) and can lead to a biased estimation of fish biomass and size distribution (Nilsson & Folkedal, 2019). Our knowledge about GS fish is poor due to the limited visibility of fish in sea cages. A promising method to monitor GS fish in sea cages is to deploy cameras and to develop automatic image processing pipelines. Recently, deep-learning-based tools, such as Convolutional Neural Networks (CNNs), have been successfully employed in marine sciences to automatically analyse video and image data (e.g. Crescitelli et al., 2020). The aim of this study is to classify the GS phenotype in a computer-vision framework by identifying differences between them and healthy fish. We report the results of the best performing classification model, which can be used in the creation of an automated counting system that will provide the abundance and distribution of GS and healthy fish in sea cages. Such knowledge can be used to understand connections between fish and environmental/production factors, which will help us to counteract factors leading to the development of GS fish.

Materials and methods

Video data was collected at a commercial Atlantic salmon farm in Norway by using underwater cameras (GoPro8) placed in a sea cage by cage walls at 4m, 8m, and 12m depths. Frames which included both GS fish and healthy fish were extracted from the videos, and 249 healthy fish, and 103 GS fish were annotated and saved as new images. The images were divided into two collections: one composed of 246 images was used for training models, while another with 106 images was used for testing. For extracting identification features, we used Resnet-101, ResNet-152, FBResNet-152, InceptionV4 and InceptionResNetV2 models which were pre-trained in the ImageNet collection[1]. Each CNN extracted feature vectors with size 2048 for each image. We also used a t-distributed Stochastic Neighbor Embedding (tSNE) method, a visualization technique for high-dimensional data that gives each point a location in a two-dimensional map. A Support Vector Machine (SVM) and a Random Forest (RF) binary classifier were then trained to classify input images as a healthy or GS fish. We also performed a grid search to determine the best parameters for SVM and RF models.

Results

The use of pre-trained CNNs led to effective representations of the visual properties of GS and healthy fish. When comparing the values of the analysed classifiers (Table 1), SVM outperformed RF in almost all of the studied cases. The only case that RF presented a better accuracy was for InceptionV4. The best results were observed when ResNet-101 model was used as feature extractor (Table 1). The classification system was based on SVM classifier, since it was the best classifier (Table 1). After performing a grid search with 5-fold cross-validation on the training set, the configuration of SVM with the best configuration was: *C = 0.1*, *gamma = 1000*, and *kernel = linear.* With this configuration, the classification system reached **97.17%** correct identification in the test set. When analysing the confusion matrix, the best model did not commit any mistakes when classifying GS fish (i.e., 100% accuracy (31/31) on this configuration for the dataset). The accuracy in classifying healthy fish was 96% (72/75). The use of *linear* kernel also corroborates with tSNE analysis, since the dispersion of the data demonstrates that they are well clustered into two well-defined groups (Fig. 1).

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Fig. 1. *Two-dimensional representation of the features of healthy (blue) and GS fish (red). Points represent the 2D projection of image ResNet-101 features. Most of the samples belonging to the two classes are well separated in the 2D space.*

Discussion

Preliminary results show that the creation of a video-based automatic system for recognition and counting GS fish is feasible. Cameras deployed inside sea cages are extremely important since we can generate a great amount of images and videos that can have their features extracted. These cameras can also monitor behaviour of fish, and GS fish can be automatically identified by using the obtained insight on GS fish features. The results show the great potential for the use of SVM in the automatic detection of GS fish, leading to a significant reduction of the human workload required for manual analysis. In our future work, we intend to use videos instead of images, and integrate environmental variables, farm operation information such as feeding strategy, and camera locations into the analyses. Such advanced analyses will improve our understanding of factors leading to GS fish occurrence and their spatial and temporal distribution patterns, which will increase fish welfare and farm production.

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BIOBASED ROPES (BIOGEARS) USE IN LONGLINE *VS.* RAFT MUSSEL (*Mytilus galloprovincialis*) AQUACULTURE: COMPARISON OF GROWTH PERFORMANCE AND QUALITY

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Introduction

Low trophic aquaculture, such as bivalve species, can be candidate species for the minimization of environmental impact of worldwide growing aquaculture, while providing consumers with high quality and nutritive seafood. Nowadays, mussels are cultured suspended mainly in raft and longline systems, using ropes made of non-biodegradable petrol-based plastic, currently estimated in 1,600,000 to 3,300,000 metres/year. The current growth estimations of mussel offshore productions would correspondingly increase the use of petrol-based plastic ropes. Inadequate waste collection facilities at harbours and end of life (EoL) management possibilities of these ropes, may result in augmenting marine litter and microplastics entering the oceans. EU BIOGEARS project, addresses the challenge of minimizing the use of petrol-based plastics in the sea by developing biobased and compostable ropes, **biogears.** At the end of life, biogears are being validated to entering in-land organic recycling circuits, hence greatly reducing carbon footprint along the whole value chain. Thus, generating new local value chains by promoting biobased circular economy. The aim of this study is to assess and compare the aquaculture performance and quality of mussel cultured suspended in two biogears prototypes vs. commercial rope counterparts in an offshore longline and in a raft.

Material and methods

Biogears prototypes of two different compounds based on commercially available biobased polymers, B1 and B2, were manufactured with currently used industrial processes for aquaculture rope manufacturing. The biogears prototypes were designed to be fit-for-purpose with adequate durability and functionality for mussel productions. Twenty-four ropes of 5m length of prototypes B1 and B2, and a commercial counterpart (GR) were seeded with juvenile mussels (9.44 ± 4.27g body weight, 53.35 ± 15.63mm shell length), at a density of 666.2 ± 89.34 individuals per meter rope (5,632.5 ± 89.34g/m rope). Seeded ropes were deployed in an experimental raft in the port of Mutriku, for a conditioning period of two months (July-Sep 2021). In September 2021, twelve ropes of each type were transported and deployed in a longline system located 2 miles offshore of the coastline, while others remained in the raft. For one production cycle (July 2021-July 2022), the rope effect on mussel grow-out in longline vs. in raft will be assessed and compared. Bimonthly, growth and survival of the mussels, condition index and meat yield, proximal composition and fatty acid profiles are being assessed in both locations (SE Bay of Biscay).

Results and discussion

Preliminary results of the mussel growth performance in the experimental period from September 2021 to March 2022 are shown in Figure 1. After the conditioning period B1 mussel showed a higher shell length and body weight than mussel from the rest of groups (B2 and GR mussel showing similar size, in body weight and shell length). Correspondingly, mussel abundance (in weight of individuals) was also higher in B1 group. Over the six months in culture the overall mussel growth was higher in longline than in raft cultured mussel, both in shell length (~ 5 mm), body weight (~ 4g) as well as in mussel abundance (~ 750 g), irrespective of the rope type. Mussel shell length was not affected by rope type either grown-out in longline or in raft after six months. However, B1 and GR mussels grown-out in longline and B1 grown in raft systems showed higher individual body weights. An overall loss of mussel occurred in all types of ropes over the experimental period. In longline, biobased ropes B1 and B2 showed higher mussel final weight per linear meter of rope than the commercial counterpart (GR). In the raft, the final abundance of mussel was higher in B1 than in the rest of groups, observing similar values among B2 and GR ropes.

Overall, these preliminary results may suggest rope type effect on mussel growth. Biobased rope B1 promoted a higher mussel growth and abundance than commercial fossil-source plastic ropes (GR) in raft. In longline, B1 and B2, promoted higher mussel abundances, which could be due to a better attachment of mussel to biobased ropes. Attachment to ropes is a critical aspect that influences mussel crop loss especially in offshore productions in high energy environments. Thus, biobased ropes could lead to more promising production yields than GR ropes in raft and especially in longline systems. Composition and quality parameters are being analyzed.

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Fig. 1. Growth performance of mussel in shell length (a, b), body weight (c ,d) and abundance (e, f) cultured in biobased B1 and B2 ropes and commercial petrol- based plastic ropes suspended in longline and raft systems.

EVALUATION OF THE SUCCESSFUL SETTLEMENT OF PURPLE SEA URCHIN (*Paracentrotus lividus*) ON SUBSTRATES COMPOSED OF SOLID AGAR MEDIUM AND DIFFERENT MICROALGAE (*Nitzschia closterium*, *Phaeodactylum tricornutum*, *Tetraselmis sp.* IMP3)

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Introduction

The settlement and metamorphosis of sea urchin larvae is undoubtedly one of the most critical stages of their life cycle. The transition from the pelagic to the benthic life form implies a radical change in the form of feeding, moving from the filtering ingestion of phytoplankton to an active scraping feeding. In the post-larvae phase, sea urchins start to feed on biofilm composed of various marine microorganisms, such as benthic microalgae, bacteria and fungi. The induction mechanism for settlement is still poorly understood. The sea urchin settlement is promoted by the presence of certain natural chemical compounds (polyunsaturated fatty acids, dibromomethane, glycoglycerolipins, among others) associated with food, and also inorganic chemical compounds such as like ionic salts. To obtain a great number of viable juveniles in sea urchin aquaculture, it is required to provide cultivating conditions that optimize metamorphosis and sedimentation success rates. This success involves the creation of substrates for settlement, previously colonized with biofilm composed of microalgae and other marine microorganisms. The settlement of *Paracentrotus lividus* on solid agar media previously inoculated with three different microalgae was analyzed in this study. The settlement rate was observed between days 20 and 27 DAH.

Materials and methods

For the evaluation of *P. lividus* larvae settlement substrates, petri dishes with solid medium composed of agar (1.5%), Guillard's F2 culture medium and silica were used. Using the agar as solid medium, 5 types of microalgae biofilms substrates were subsequently developed: *Nitzschia closterium* (*Nc*), *Phaeodactylum tricornutum* (*Pt*), *Tetraselmis sp.* IMP3 (IPM3), a mixture of the 3 microalgae (*mix*) and a control (*Ctrl*) without microalgae. For inoculation on the agar substrates, microalgae cultures were spread using the following densities: 2.4 x10^6, 7.5 x10^6, 4.4 x10^6 cell/ml, for *Nc*, *Pt* and *Timp3*, respectively.

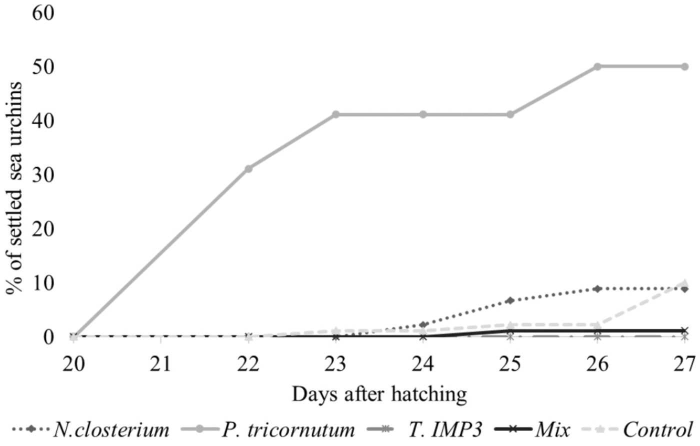

Figure 1- Cumulative percentage of sea urchins (*Paracentrotus lividus*) fixed on the different substrates analyzed *Nitzschia closterium*, *Phaeodactylum tricornutum*, *Tetraselmis sp.* IMP3, mix and control.

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In order to obtain *P. lividus* gametes, 1 ml of 0.5 M KCl was injected into the coelom via the peristomal membrane. Fertilized eggs were hatched in a 220 L cylindrical fiberglass tanks with soft aeration and covered with shader to reduce environmental light exposure for approximately 48h. The larval cultivation experiment was carried out in 6L flat bottom flasks without water inlet and with a reduced aeration, with a density of 2500 larvae per liter. During this period the larvae were fed a mixture of 2 microalgae: *Isochrysis galbana* and *Skeletonema costatum*. Periodically, observations were made in a stereomicroscope to follow the larval development. A selection of larvae suitable for the settlement procedure was undertaken on day 20 DAH (rudiment diameter equal to or greater than the diameter of the stomach). For each treatment, 3 petri dishes were used in which 30 competent larvae were introduced, in 40 ml of sterilized sea water. The petri dishes were kept in a phytoclimate chamber at a temperature of 19ºC and a photoperiod of 12 h of day light. An observation and counting of settled larvae were performed every day. Daily, 20% of the culture volume was replaced with sea water containing the planktonic microalgae *S. costatum* (settlement larvae feeding) at a density of $2.5x10^5$ cells/ml.

Results and discussion

When comparing the development of the 3 microalgae in the solid agar medium, there was a lower growth in diatoms (*N. closterium* and *P.tricornutum*) comparing to *Tetraselmis sp*. IMP3. This result was already expected, and Timp3 was chosen due to its great capacity to grow on solid media. Despite being minor, the growth of other microalgae was significant. Analyzing the results of successful settlement, it was found that on day 22 DHA the settlement process began, despite occurring only on substrates with the microalgae *P. tricornutum* (figure 1). In the following days, there was also a gradual settlement in some other treatments, being, however, much more significant in the microalgae. At the end of the test (27 DAH) there was an average settlement rate of 50.0 (± 36.6) % on substrates with *P. tricornutum*. The mean test diameter at this day was 0.33 (± 0.06) mm. For the substrate with *N. closterium* this rate was 8.8 (± 1.9) %. There was no settlement of sea urchins in the substrate with *Tetraselmis sp*. IMP3. On solid substrates without inoculated microalgae, there was a settlement rate of 10 (± 11.5) %. This result shows that benthic diatoms have an advantage over *Tetraselmis sp*. IMP3. The presence of larvae settled in the uninoculated substrate (*control*) suggests two hypotheses still under evaluation: 1) Properties and/or ecological strategies of the *Tetraselmis sp*. IMP3 may suppress the settlement of sea urchins; 2) Verified presence of other marine microorganisms developed in the *P. tricornutum* substrate contributes to a richer and suitable biofilm for the settlement and first feeding of sea urchins. At the present time, microbiological analyzes are being carried out to better characterize the developed biofilm and understand the degree of importance of microorganisms associated with the microalgae substrate. The great variance between the results of the same treatment suggests the influence of other factors besides the type of algae included in the substrate, from handling to the quality of the larvae. However, the effect of microalgae type is clearly significant on the success of settlement.

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GROWTH PERFOMANCE OF *Phallusia mammillata* (CHORDATA, ASCIDIACEA) AS BIOREMEDIATOR UNDER AN INNOVATIVE IMTA IN THE MAR GRANDE OF TARANTO (IONIAN SEA, ITALY)

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Introduction

Since the last twenty years aquaculture sector has been recognized for its essential role in providing nutrition and employment to millions of people worldwide (FAO, 2022). Yet, such industry is not without criticism as its negative environmental impacts deriving from production wastes (Wang et al., 2020), and more sustainable farming methods should be developed. Integrated Multi-Trophic Aquaculture (IMTA) is a polyculture method combining fed organisms (e.g., fish) with extractive organisms or "biofilters" (e.g., suspension-feeders, seaweed), with the aim to create a balanced system for both environmental and economic sustainability (Barrington et al., 2009). In such system fish wastes (i.e., uneaten feed, faeces) are recycled via biofiltration (bioremediation) becoming food for extractive species whose biomass could be exploited as valuable by-product, promoting circular economy (Barrington et al., 2009). So far, edible bivalves and seaweed are almost the only organisms cultured as bioremediators, and experimentation involving other efficient extractive species with valuable biomass is thus desirable.

Ascidians are benthic suspension-feeders, colonial or solitary; widely distributed worldwide and often representing the dominant component of fouling communities in eutrophic environment (Ordóñez et al., 2013). Their tolerance to eutrophic conditions and high filtration rate (Randløv and Riisgård, 1979), combined with their fast-growing rate, nutritional value and potential as source of several bioactive compounds make ascidians ideal extractive organisms to be exploited in IMTA systems (Marques et al., 2022).

In the present work, we report preliminary results over a four-month period of the growth performance of *Phallusia mammillata* (Cuvier, 1815) as bioremediator under an innovative IMTA system developed in the Mar Grande of Taranto

Materials and methods

The study area is located in the Mar Grande of Taranto (40°25′56″ N; 17°14′19″ E) (Ionian Sea), which is a semi-enclosed basin connected to the Gulf of Taranto. The investigation was performed in the aquaculture plant "Maricoltura del Mar Grande (MMG)", partner of the Remedia-Life project hosting the innovative IMTA system. Here, 42 natural fiber ropes (10 m in length) were placed around fish cages in November 2021 and used as fouling collectors to monitor the growth of *P. mammillata* for 1 year. Between March and July 2022, 3 collectors were randomly sampled monthly, and all the individuals of *P. mammillata* were counted and measured in length. Mean values of length and linear density were computed to evaluate the growth of *P. mammillata* over time.

Results

P. mammillata had a linear growth trend showing the maximum value of length in July 2022 (Fig. 1a). Linear density showed a great increment between March and April 2022 settling at about 1 individual·m^{-1} in July 2022 (Fig. 1b).

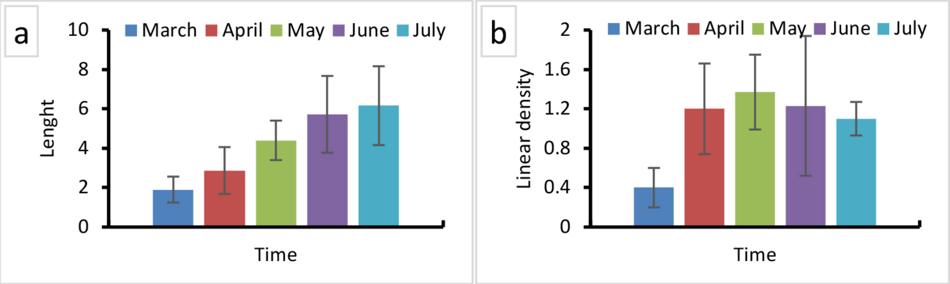

Figure 1. a) Length (cm) and b) linear density (n. individuals·m^{-1}) over time of *P. mammillata*.

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Discussion

P. mammillata has performed well under our innovative IMTA system showing in only 4 months an average length increment (224.21 ± 133.08 %) greater than *Sabella spallanzanii* Gmelin, 1791 and *Sarcotragus spinosulus* Schmidt, 1862 cultured at the same conditions (Giangrande et al., 2020), and only few specimens were not survived. Beside March 2022, linear density was quite constant throughout the sampling period since new recruits replacing any lost specimens were observed each month. Such observation is likely related to the higher temperature enhancing ascidian gametogenesis in Spring-Summer in the northern hemisphere (Millar, 1971). Thus, considering all the collectors placed around fish cages, in July 2022 we obtained by natural recruitment potentially about 450 individuals of *P. mammillata* long on average 6.16 ± 2.01 cm. This biomass obtained as byproduct represents an added value for IMTA farmers since ascidians are sources of natural compounds as, for example, the tunicin, a cellulose-like polysaccharide, or numerous molecules with antimicrobial activity to be exploited in several fields (Marques et al., 2022). In addition, the tissues of solitary ascidians have a great nutritional value with high-protein content and a fatty-acid profile similar to fish oil (Hassanzadeh, 2014), making them a good alternative to replace fish oil in the formulation of innovative fish fodder.

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IN VITRO AND IN VIVO EVALUATION OF INDUCED *Vibrio splendidus* TOWARD IMMUNE RESPONSE AND CROSS PROTECTION IN *Oyster grassostera gigas*

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Vibrio splendidus is an oyster pathogen associated with the summer mortalities affecting the production of *Crassostrea gigas oysters* worldwide. The increasing experimental evidence suggests that there are some forms of specific acquired immunity in invertebrates, but the underlying mechanism is not fully understood. In the present study, Pacific oyster (*Crassostrea gigas*) stimulated primarily by exposure to 10^5 *Vibrio splendidus* displayed stronger immune responses levels when they encountered the secondary challenge of 10^8 *V.splendidus*.we tested *Vibrio* primed by exposure to colonized in oysters to investigate their resistance against hemolymph in vivo and in vitro experiments. we investigated the immunological responses in oysters and whether this colonization results in elevated mortality.The selected strains showed higher survival in seconde challenge in oyster model and cross- protection against *Vibrio splendidus* in vitro. A population of oyster selected for resistance to the *Vibrio splendidus* showed increased in vivo survival, in addition, *V.Splendidus* reduction of pathogen shedding. In conclusion, our results suggest that the primary stimulation of *V. splendidus* led to immune priming in oysters when encountering the secondary challenge with *V. splendidus*, in addition, the increased survival of oysters almost certainly by stimulating the immune system in hosts led to prevent vibriosis in the oyster.

IDENTIFICATION OF *Vibrio kanaloa* IN THE *Oyster crassotrea gigas* BY FLUORESCENT IN SITU HYBRIDIZATION

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Background
An ideal model for studying *Vibrio* and host and their interaction in disease dynamics is *Crassostrea Gigas* in the North Sea, exposing invasive sources to pathogens, especially (*vibrio kanaloae*).

Methods
This study used molecular fluorescent in situ hybridization (FISH) techniques to rapidly identify the diversity of bacteria in the oyster *Crassostrea Gigas* from Sylt Island and 16SrRNA gene sequence validated all strains' identification. Oysters were collected and exposed with *vibrio kanaloae* strains on different tissues were examined utilizing culture-independent methodologies.

Results
the digestive glands, gill and Muscle in *vibrio Kanaloa* were identified as metabolically active by the FISH technique, one of the advantages of the FISH technique is that it is accurate and very easy to use.

Conclusions
This technique allows rapid assessment of *Vibrio* in oysters and seafood.**=**

CHARACTERIZATION OF TOXIN GENES ANTIMICROBIAL SUSCEPTIBILITY OF *Staphylococcus aureus* ISOLATES IN FISHERY PRODUCTS IN IRAN

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Introduction:
Staphylococcus aureus is one of the most common causes of seafood-borne diseases worldwide, which are attributable to the contamination of food by preformed enterotoxins.

Purpose:
The final aim of this study was to investigate the contamination of fishery products before their purchase and consumption.

Methods:
In this study, a total of 206 (34.3%) Staphylococcus aureus strains were obtained from 600 fish and shrimp samples and were tested by PCR for their antimicrobial susceptibility. We assessed the prevalence of the genes responsible for the staphylococcal enterotoxins (SEA, SEB) and toxic shock syndrome toxin 1 (TSST-1) genes.

Results:
The results indicated that 34% of aqua food samples were contaminated with S. aureus, and 23.8% of these isolates were mec-A-positive. Sixty-four percent of the strains isolated from contaminated seafood was enterotoxigenic S. aureus, and 28.2% of SEs were MRSA-positive. The most prevalent genotype was characterized by the presence of the sea gene (45.2%), followed by the seb gene (18.5%), and the tst gene encoding TSST-1 was found in eight strains (3.9%). Of the 206 S. aureus isolates, 189 strains (84.9%) were resistant to at least one antibiotic.

Significance:
Given the frequent outbreaks of enterotoxigenic MRSA, it is necessary to make revisions to mandatory programmes to facilitate improved hygiene practices during fishing, aquaculture, processing, and sales to prevent the contamination of fishery products in Iran.

ANTIBACTERIAL ACTIVITY OF THE SEA CUCUMBER *Holothuria leucospilota* WHOLE BODY EXTRACT AGAINST METHICILIN RESISTANT AND ENTERTTOXIN PRODUCING *Staphylococcus aureus* STRAINS

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Introduction:
Preformed enterotoxins of *Staphylococcus aureus* are one of the most common causes of seafood-borne food poisoning worldwide. Aquatic organisms, including those used as seafood, are also a source of organic compounds of potential medical importance.

Purpose:
Within the framework of an antimicrobial activity study of marine macro-organisms from the Persian Gulf, bioactive compounds of the sea cucumber *Holothuria leucospilota* were extracted from whole sea cucumber bodies using chloroform or methanol. The extracts were evaluated for their antibacterial effects against methicillin-resistant *Staphylococcus aureus* (MRSA) and enterotoxin producing *Staphylococcus aureus strains* (SEASA, SEBSA).

Methods:
Antimicrobial activities were determined using three methods: disk diffusion tests, minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC).

Results:
The results demonstrate that methanol and chloroform extracts have an inhibitory effect on the growth of all strains tested at MIC concentrations up to 100 mg/ml. Also, the chloroform extract demonstrated bactericidal activity against SEBSA in concentrations of about 100 mg/ml. The extract below 100 mg/ml concentrations also shows bactericidal effects against MRSA and SEBSA. The highest antibacterial activity was found in the methanol extract.

Significance:
Therefore, sea cucumber extract is an interesting candidate for the identification of new antimicrobials, however, comprehensive investigations are needed to separate and identify the active compounds from *Holothuria leucospilota* from the Persian Gulf.

GENETIC VARIATION, GWAS, AND PREDICTABILITY FOR SURVIVAL AGAINST IPNV STRAINS IN RAINBOW TROUT

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Introduction

Contagious diseases are a major threat in aquaculture due to losses caused by high mortalities and the reduced growth of surviving fish. Infectious pancreatic necrosis (IPN) is one of the highly contagious diseases of farmed salmonid fish caused by *Aquabirnavirus*. The disease often causes high levels of morbidity and mortality (30–80%, [1]), and ultimately huge economic loses. Juvenile fish and post-smolts during the months following seawater transfer appear to be the most susceptible phases of production cycle. The clinical symptoms of disease outbreak include swollen abdomen and eyes, darkening of the skin, necrosis of the pancreas and spiral swimming [2]. The survivors can become healthy carriers that may infect susceptible animals either by vertical and/or horizontal means. Although a large proportion of rainbow trout are vaccinated against IPN-virus, the protective effect is uncertain. Host genetic make-up plays significant role for survival against infectious pancreatic necrosis virus (IPNV) with some families survive better than the others. Genetically improved resistance against IPNV is a highly valuable tool to improve survival and to reduce losses due to IPN. The aim of current study was to compute genetic variation for survival against IPNV, further look into the genomic architecture of the trait and explore potential for marker assisted and/or genomic selection.

Material and Methods

The population used in current study originated from the breeding nucleus (year class 2021) of Osland Stamfisk AS, comprising 98 full-sib families. Population was created as crosses among 61 sires and 75 dams, with an average family size of 23 sibs per family (N=3 to 47). Challenge test for survival to IPNV was performed at fry stage with immersion method using two different strains of infectious pancreatic necrosis virus: rainbow trout specific strain of IPNV (IPNV-RT) and Atlantic salmon specific strain of IPNV (IPNV-AS). The IPNV strain was obtained from a rainbow trout farm during field outbreak, and IPNV-AS strain was a previously available isolate. The fry were challenged in two tanks, where one tank (E-4, IPNV-RT) had fry from all 98 families, while the other (E-5, IPNV-AS) had fry from 25 full-sib families. The challenge test model had been previously established and optimized by VESO Vikan, where the concentration of IPNV-RT virus in tank E-4 was set to 6.8 x 10^7 $TCID_{50}$ per ml while IPNV-AS virus in tank E-5 had a concentration of 1 x 10^8 $TCID_{50}$ per ml. Survival phenotype was recorded during the challenge test and all the surviving and dead fish were tissue sampled for genotyping to construct pedigree and for the later genomic analyses.

Analyses: Estimation of genetic parameters, genome-wide association analysis (GWAS), and predictions of estimated breeding values (EBVs) were performed using a linear mixed model(s). Estimates of genetic parameters were computed using ASREML v4.2 applying genomic or pedigree information, and the GWAS analysis was performed with GCTA using "--mlma-loco" function [3]. Moreover, the power of predictability was evaluated using cross validation scheme with different models (PBLUP, GBLUP and Bayesian) to assess and compare the potential of genomic and/or marker assisted selection over classical pedigree information.

The applied model and components are explained below,

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

Where y is the vector of phenotypes (0 dead or 1 alive); μ is the overall mean; $\boldsymbol{Z}$ is a design matrix to relate the records to genetic values and u is a vector of random additive genetic effects, it is assumed that $u \sim (0, \sigma_g^2[\mathrm{A/G})$, where $\sigma_g^2$is the genetic variance, $\boldsymbol{A}$ is the relationship matrix obtained using pedigree information, $\boldsymbol{G}$ is a genomic relationship matrix computed using VanRaden's method 1. The $\boldsymbol{e}$ is the vector of random residual effects with $e \sim N(0, I\sigma_e^2)$.

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Results and Discussion

The obtained heritability estimates for survival to IPNV-RT were 0.33±0.12 and 0.27±0.03 using pedigree vs. genomic information, respectively. These estimates for survival to IPNV-AS were 0.16±0.04 and 0.20±0.06 with pedigree and genomic information, respectively. However, the estimates derived for survival to IPNV-AS are indicative estimates because these are obtained from small set of families (n=25). The bivariate animal model revealed strong genetic correlation for survival to IPNV-RT and IPNV-AS with estimates of 0.85±12. As, genetic correlation was high and therefore general survival as a single trait was also used to obtain estimates. The general survival showed heritability of 0.25±0.03.

The accuracy of predictions and GWAS analyses were performed for each trait separately. However, GWAS and predictability results obtained through general survival are described as follows. The GWAS analysis revealed a strong signal of QTL at chromosome 1 comprising 23 SNPs presenting significant association to the survival trait with P-value crossing genome-wide Bonferroni corrected threshold (Figure 1) The proportion of the genetic variance explained by the highest significant SNP was up to ~27% of the total genetic variance. The genomic Bayesian models performed the best in terms of predictability with ~21% improvement in predictability compared to PBLUP model.

In conclusion, survival to IPNV-RT and IPNV-AS in current population revealed significant genetic variation with one major QTL contributing significant proportion of genetic variation while remaining contribution possibly coming from many other loci. The prediction using genomics-based Bayesian models outperforms the predictions using PBLUP model.

Acknowledgement

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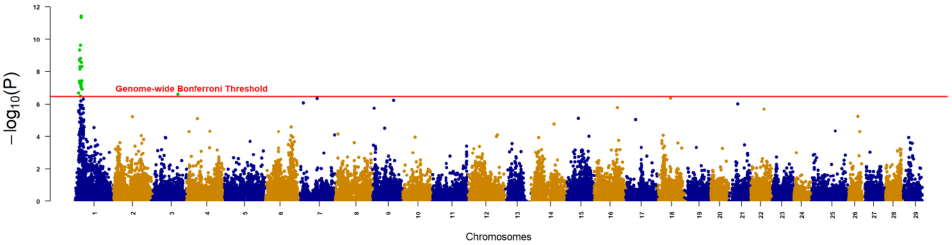

Figure. 1: Manhattan plot presenting association of SNPs with the survival trait.

CAN BABY SPINACH (*Spinacia oleracea*) IN AN AQUAPONIC SYSTEM SPEED UP THE NITRIFICATION PROCESS IN MATURING BIOFILTER AND REDUCE THE OFF-FLAVOR COMPOUNDS?

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Introduction

Aquaponics is a farming method where the nutrient-rich wastewater from a recirculating aquaculture system (RAS) is utilized to grow plants. In an aquaponic system, the ammonia produced by fish metabolism is oxidized by biofilter nitrification microbes to nitrate which is an essential nutrient for the plants but is also well tolerated by fish. Fish feed contains also other nutrients that are important for plants. It is important to manage the water quality in an aquaponics system and to maintain the optimal growing conditions for fish and plants. Fishes thrive in clean water while the plants require nutrient-rich medium for their growth. In RAS cyanobacteria, *Actinomycetes* and myxobacteria are known to produce off-flavor compounds geosmin (GSM) and 2-methylisoborneol (MIB) which can easily accumulate in fish flesh resulting in earthy and musty flavor and making them unmarketable. Thus, the removal of these compounds by depurating them in flow-through water is necessary before marketing. The aims of this study were to investigate the growth of baby spinach (*Spinacia oleracea*) and rainbow trout (*Oncorhynchus mykiss*) and to compare water quality in RAS, hydroponics (i.e. soilless culture of plants) and in a coupled aquaponic system. Special emphases were given to off-flavor compounds in fish flesh and plants as well as the onset of nitrification process in RAS and aquaponics.

Materials and methods

A six-week experiment was performed with three replicated RAS, aquaponic and hydroponic systems. Twenty rainbow trout (initial weight 108 g) were stocked in each of the six fish tanks: three for RAS and three for aquaponic systems. Twenty-five baby spinach **(*Spinacia oleracea*)** plants were transplanted in each of the six deep water culture rafts (3 for aquaponics and 3 for hydroponics). Biomass for the fish and plants were recorded in the beginning and at the end of the experiment. Chloride, phosphate, sulfate, nitrate, nitrite, GSM and MIB were compared between RAS, aquaponics and hydroponics at the start and end of the experiment. The water quality data (total ammonia nitrogen, nitrite, nitrate, and pH) were collected during the experiment and the nitrification process was compared between RAS and aquaponics treatments.

Results

The fish grown in aquaponics exhibited statistically higher weight (137.1 ± 11.29) compared to fish grown in RAS (109.3 ± 3.05). Specific growth rates were also higher for fish grown in aquaponics (1.95 ± 0.12) than in RAS (1.67 ± 0.08). The feed conversion ratios (feed fed/weight gain) were lower for the fish grown in aquaponics (0.86 ±0.08) than in RAS (1.06 ±0.03). The concentrations of GSM were lower in fish flesh grown in aquaponics (376.0 ± 24.99) than in RAS (466.3 ± 39.40). The concentrations of MIB were at similar levels in fish flesh grown in aquaponics and RAS. The concentrations of GSM and MIB did not differ statistically significantly between the water of the two systems. The onset of nitrification was faster in the aquaponic system than in RAS (Figure 1).

In the aquaponics system, the plant dry weight of spinach was 43 % higher (40 % for shoot and 70 % for root) than in hydroponics. The concentration of GSM was significantly higher in the roots of spinach grown in aquaponics (3579 ± 1682.8 ng/L) compared to spinach grown in hydroponics (191.80 ± 48.78 ng/L), but in the shoots the level of GSM was very low in both treatments (~8 ng/L). The concentration of MIB was higher in the shoots of spinach grown in aquaponics (704.4 ± 73.08 ng/L) than in those grown in hydroponics (278 ± 158.2 ng/L).

Conclusions

The aquaponic system was better than RAS in terms of water quality, onset of nitrification, fish growth and feed conversion ratio and also lower concentrations of GSM accumulated in fish flesh. Spinach grew better in aquaponics than in hydroponics but the difference was not statistically significant.

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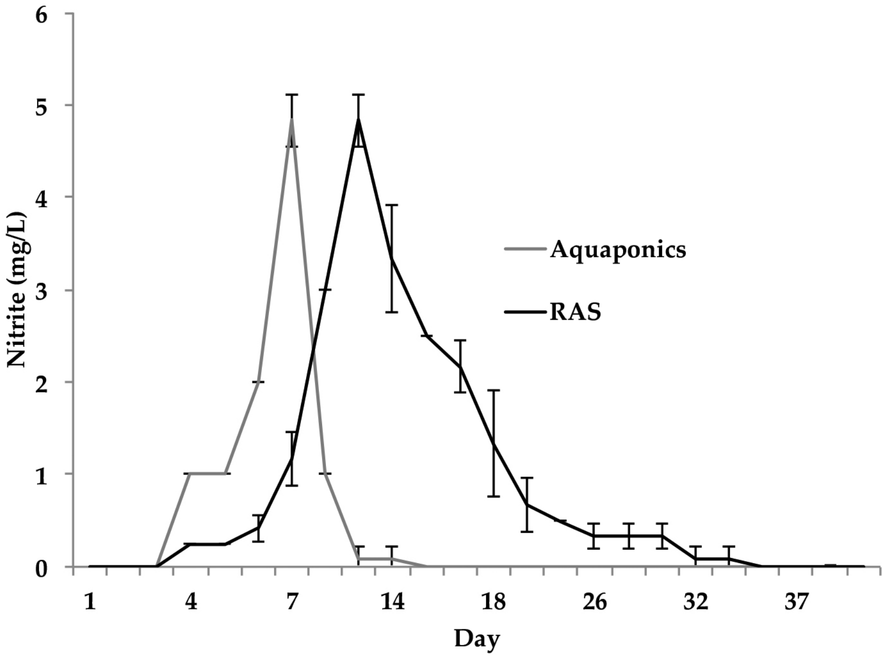

Figure 1. The mean concentration (mg/L) of nitrite in RAS and aquaponics treatments during the 42-day experiment. For the aquaponics treatment spinach was grown in a coupled aquaponics system together with rainbow trout (*Oncorhynchus mykiss*). Repeated measure ANOVA, ± SD (n=3).

Acknowledgments: We would like to thank University of Jyväskylä, JAMK University of applied Sciences, Sisä-Suomen kalatalousryhmä, Maa- ja Vesitekniikan Tuki Ry, Niemi-säätiö and Luonnonvarakeskus (Luke) for their contribution towards this research work.

ENVIRONMENTAL PERFORMANCES OF SEABREAM-OYSTER-CLAM-SHRIMP IMTA: THE LIFE CYCLE ASSESSMENT POINT OF VIEW

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Introduction

Integrated Multi-Trophic Aquaculture (IMTA) is a promising way to improve the sustainability of aquaculture systems, by a better use of the nutrients provided, using the trophic complementarity of several aquaculture species (Neori et al., 2004). However, there are still lack of references and knowledge on the real environmental performances of such systems as the addition of supplementary species may induces supplementary impacts (Nederlof et al. 2021). To better understand the multiple environmental consequences of changes of practices, Life Cycle Assessment (LCA) is a key method (Aubin et al, 2015). However in LCA, data collection and methodological points (functional unit, allocation rules…), are still obstacles in complex systems such as IMTA. In our study, we applied LCA to a seabream-oyster-clam-shrimp experimental IMTA conducted in coastal ponds during two rearing periods, and compared with the practices of a private farm producing oysters-clams and shrimp in similar structure but at a higher production level.

Material and methods

This study was conducted in the framework of the EU-Prima SIMTAP project. The experiment was conducted at the Lycée de la Mer et du Littoral (Charentes Maritimes, France), in coastal ponds usually dedicated to production and refinement of oysters. Four ponds (400-700 m^2) were associated in a loop using pumping and spillway. The first pond was dedicated to the rearing of 200g seabreams (*Sparus aurata*), fed with vegetable feed and discarded mussels (*M. edulis*), while the three downstream ponds were dedicated to the growth of mixed oyster (*Crassostrea gigas*), clam (*Ruditapes philippinarum*) and shrimp (*Marsupenaeus japonicus*). The water is sent back to the first pond, after the last pond of oyster-clam and shrimp. Additional ventilation is provided by airlift in each pond.

The experiment was conducted from June to October in 2020 and 2021. After the first year, considering the survival rate and the flesh yield of the shellfish the seeding of clams and oysters was increased. Between the two years, there were also variations in survival in the ponds due to climatic or handling incidents. The resulting production is indicated in table 1. We compared the performances with a commercial farm of 30 ha producing a mix of oyster, clam and shrimp, using formulated feed to sustain the worm productivity of ponds, for the shrimp feeding. The average production performances of the farm are presented in table 1.

LCA was conducted from cradle to farm gate, and calculated with different functional units (results presented by tonne of aquatic products), using SimaPro 9.3 software and Ecoinvent 3.8 and AgriBalyse databases. Four impact categories are presented here: Climate Change (kg CO_2-eq), Acidification (kg SO_2-eq), Cumulative Energy Demand (MJ), Eutrophication (kg PO_4-eq), Land competition (m^2.y).

Results and discussion

In the two SIMTAP trials, the rearing performances were good, with expected growth of seabreams with the innovative feed formula, very high survival rate (~95%) in oyster and very high flesh yields (over 20%), expected survival rates and high flesh yields in clam, and expected survival rates and good growth in shrimp. However, the position order of the pond in the loop induced variation in growth and survival for shrimp, oyster and clam.

The trial SIMTAP 2020 induced the highest levels of all environmental impacts, while SIMTAP 2021 induced the lowest levels in Climate Change, Acidification and Land competition. The Commercial farm induced the highest level in Climate Change, and the lowest level in C. Energy Demand, Eutrophication and Land competition (Fig.1). The analysis of the contribution of the different parts of the system explains these results. First, LCA is very sensitive to production yields. The increase in production of 45% between 2020 and 2021 induced a decrease in the influence of the fixed “environmental costs”, especially the land use and the electricity (for pumping and air supply) which remained at the same level. The electricity consumption was a major impact contributor in the SIMTAP system when compared with the Commercial

(Continued on next page)

Table1: Annual production in kg of the different IMTA systems studied

	Seabream	Shrimp	Oyster	Clam
SIMTAP 2020	418	42	120	92
SIMTAP 2021	443	54	325	151
Commercial Farm		3600	7200	7200

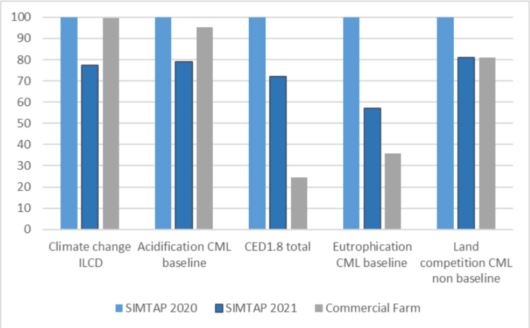

Figure1 : Comparative environmental impacts calculated by LCA, of 1 tonne of aquatic products in IMTA systems : seabream-oyster-clam-shrimp (SIMTAP) in 2020 and 2021, and oyster-clam-shrimp in a commercial farm

farm, which doesn't use pumping for water renewing (water renewal by the tide). Another large difference between the SIMTAP and Commercial farm was the contribution of the large seabreams (200g) entering the system, however it was partially balanced by the lower feed impact in SIMTAP, which leaded to lower Climate change in SIMTAP 2021 than in Commercial farm. Eutrophication due to the emissions of nitrogen and phosphorus in water was also influenced by the pre-grown seabreams.

Conclusion

The SIMTAP has very good potential, as it is productive and permits to reach good environmental performances. However, it is not optimized. The rearing performances show that there are room to increase the production of shellfish. LCA pointed out the question of electricity use, which should be mitigated by the use of renewable energy sources such as solar panels or wind turbines. The size of the seabream entering the system, chosen for their ability to use discarded mussels, has to be questioned in relation to the feeding strategy. Other impact categories are needed in this study to complete the environmental performance analysis, as well as economic and social indicators to compete the sustainability assessment.

As presented here, IMTA seems to be an interesting perspective for aquaculture. However, it is the details of application modalities that will make possible to achieve the expected environmental gain.

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PROVIDING NEW SOLUTIONS OF FISH STOCKING FOR FRESH WATER POND SYSTEMS IN FRANCE

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Introduction

Freshwater ponds are agroecosystems that provide important ecosystem services (ES) to landscapes, as well as nature's contribution to people (NCP), in particular by hosting recognized biodiversity (Blayac et al., 2014; Rey Valette et al., 2022). These ES/NCP can be influenced strongly by management practices. In France, the low profitability of freshwater ponds for fish production runs the risk that they will be abandoned, and thus that the ES/NCP that they provide will disappear. Moreover, pond management practices in France are poorly documented, as are their influence on pond productivity and provision of ES. Our study aims to recommend new fish stocking practices to meet fish farmers' expectations and better understand relations between management practices, biodiversity and ES/NCP.

Materials and methods

The SEPURE project is funded by the European Maritime and Fisheries Fund. It gathers scientists from various backgrounds (aquaculture, environmental sciences, modeling, ecology) to relate farmer practices, economic and environmental performances and the response of pond ecosystems by monitoring water quality, phytoplankton, zooplankton, benthic macroinvertebrates, aquatic macrophytes and fish populations.

After gathering available stocking and fishing data, a survey was performed and meetings were organized with fishpond farmers in three emblematic pond areas in France: Dombes, Lorraine, and Brenne-Sologne. Based on analysis of the contexts and the farmers' expectations, fish stocking scenarios were developed. At 10 production sites, farmers applied these scenarios, and the practices and ecosystems were monitored. Performances of experiments in 2021 are being analyzed, and a second year of experiments has begun in 2022.

Results and discussion

Eleven scenarios with different mixtures of species were co-designed with the fish farmers. They considered many parameters, such as the complementarity of species based on their trophic and behavioral traits (Lecocq et al., 2019), water availability, water temperature in summer, the existence of local and national markets for the species, the presence of submerged vegetation, pond depth, feeding and liming practices and the use of ponds for other activities (e.g. hunting).

Thus, the scenarios of fish stocking were diverse and covered several objectives, such as maximizing carp production, increasing profitability, allowing aquatic plants to develop to increase habitat availability for wildlife, and applying Chinese food web concepts…As mentioned, 10 of the 11 scenarios were applied in the three regions. As an application, the performance of one pond in Dombes and one in Brenne will be presented, since they differed greatly in term fish stocking and biodiversity. The results for productivity, revenue, biodiversity and environmental impacts will be discussed.

Conclusion

Revitalizing the pond sector in France is challenging. Doing so requires creating and organizing a large dataset to have a comprehensive and multicriteria vision. It is also necessary to validate approaches by applying them in the field in order to assess real-world consequences of management choices. The influence of fish production's sustainability and biodiversity conservation on ES/NCP needs to be assessed to be able to develop agroecological management of fishponds. This information should help support decision making for future development of the pond sector and help maintain local economies in these pond landscapes.

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DELINEATING THE AREAS OF A LAKE THAT ARE SUITABLE FOR TILAPIA CAGE FISH CULTURE

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Introduction

We delineated areas that have different levels of suitability for use as tilapia cage culture sites in the Kenyan part of Lake Victoria, Africa. The lake hosts one of the largest freshwater fisheries in the world, providing a significant source of protein in East Africa and exporting fish to the European Union, United States, China and Japan. The study area was 4,100 km^2.

Methodology

The method uses high-resolution bathymetric data, newly collected water quality data from all major fishing grounds and cage culture sites, and existing spatial information from previous studies. The spatio-temporal (space and season) parameters considered are water depth, water temperature, levels of dissolved oxygen, chlorophyll-*a* concentrations, distances to the lake shoreline and proximity to other constraints on cage culture development.

Results

The results indicated that the area most suitable for fish cages comprised about 362 km^2, or approximately 9% of the total area; the remaining 91% (i.e. 3,737 km^2) was found to be unsuitable for tilapia cage culture (Figure 1). We conclude that the successful implementation of this approach would need stakeholder involvement in the validation and approval of potential sites, and in the incorporation of lake zoning into spatial planning policy and the regulations that support sustainable use while minimising resource use conflicts. The results of this study have broader applicability to the whole of Lake Victoria, and other Great Lakes in the world for tilapia cage culture.

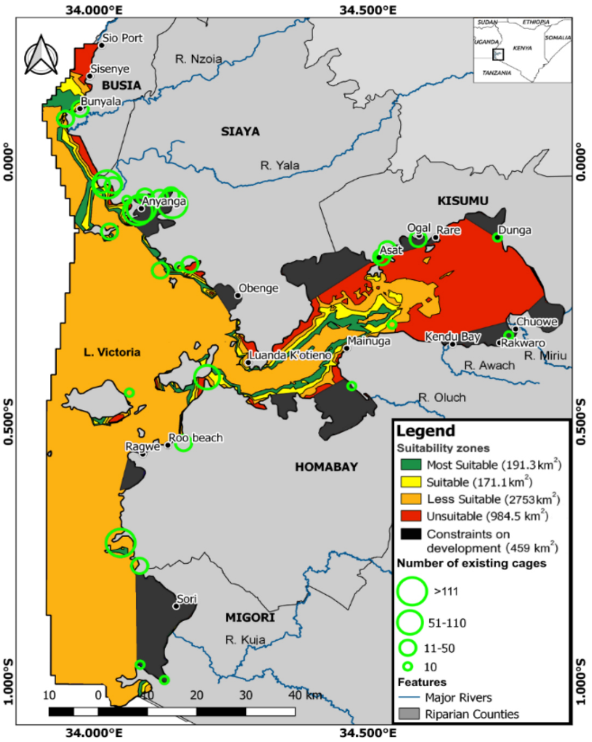

FIGURE 1 Map of Lake Victoria, Kenya, showing potential suitability for cage fish culture.

THE RELATIONSHIP BETWEEN REARING WATER MICROBIOMES AND FISH MICROBIOMES IN RAS FOR SMOLT PRODUCTION: IMPACT OF MEMBRANE FILTRATION AND ORGANIC LOADING

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Introduction

Successful fish production in RAS depends on microbes: First, microbial conversions of toxic ammonia in the biofilter is fundamental for maintaining a good chemical water quality. Second, the fish is exposed to the complex microbial communities suspended in the water. For optimized fish production, a good *microbial water quality* must be ensured, i.e., detrimental fish – microbe interactions must be prevented by selecting against rapid-growing, opportunistic bacteria in the system. We have previously shown that this can be obtained in RAS[1,2]. The interactions between the microbiota suspended in the rearing water and the gut and skin microbiota of the fish is however poorly understood. We recently showed that membrane filtration in RAS with Atlantic salmon parr reduced the TOC levels and bacterial blooms in a period with high organic loading[3]. In the present study[4], we investigated the gut microbiota of parr of Atlantic salmon from the same experiment, i.e., during periods with varying organic loading in two RAS; one with, and one without membrane filtration. The aim was to elucidate the complex relationship between the bacterial communities in the rearing water and the fish gut, and to evaluate the use of membrane filtration to promote positive fish–microbe interactions.

Materials and methods

Two pilot-scale RAS, one with membrane ultrafiltration of a 10–15% side-stream (mRAS), and one without (cRAS), were operated with Atlantic salmon parr in periods with different organic loading and degree of recirculation (previously described[3]). In the current study we focused on the two last periods of the experiment, Period 2 (P2; day 19–51) and Period 3 (P3; day 52–72). P2 was characterized by high water exchange rate and normal feed load, whereas P3 was characterized by moderate water exchange rate and high feed load. Samples were taken at the end of these to periods for characterization of gut and rearing water microbiomes.

Results and discussion

The rearing water microbiomes differed both between systems (cRAS vs. mRAS) (PERMANOVA $p = 0.002$) and sampling times (Fig. 1A). In cRAS, the water microbiome was more influenced by the high organic loading (during P3), with high abundances of two OTUs representing *Mycobacterium* (OTU_4) and *Spartobacteria* (OTU_6).

The gut microbiomes also differed significantly between cRAS and mRAS (Fig. 1b, PERMANOVA, $p=0.006$ (D50) and $p=0.0001$ (D72)), and gut microbiomes changed considerably more in cRAS than mRAS during P3 (high organic load).

The rearing water and gut microbiomes were generally highly dissimilar, but the similarity increased considerably in cRAS after P3 (Fig. 2a). Analysis of OTU abundances revealed that most OTUs were abundant either in water or gut samples. OTU_4 and 6 stood out as being highly abundant in both water and gut samples (Fig. 2b), and apparently represented bacterial populations that were able to colonize both the rearing water and the fish gut.

Conclusion

Rearing water and gut microbiomes were highly dissimilar, but high organic loading in cRAS resulted in growth of opportunistic bacteria that were able to colonize the fish gut. This led to a higher similarity between water and gut microbiomes. The present study further shows that membrane filtration is an applicable strategy to counteract growth of opportunistic bacteria in RAS and to promote positive fish – microbe interactions.

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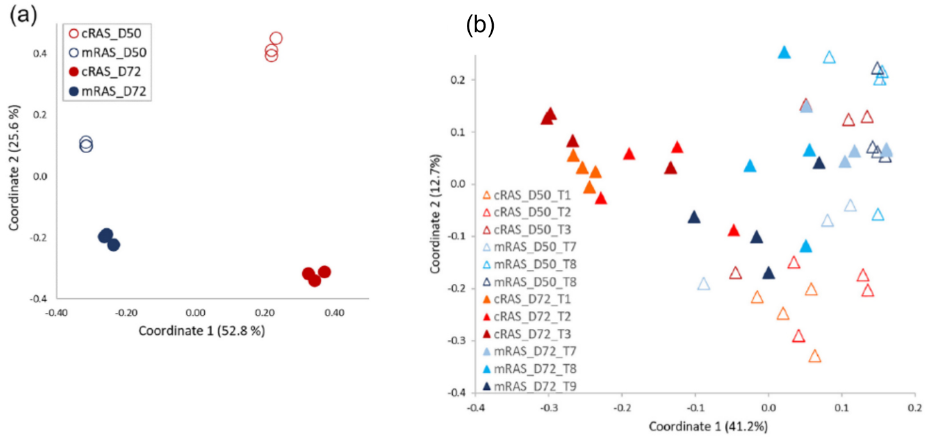

Figure 1. PCoA ordination based on Bray Curtis similarities for comparison of the microbiomes associated with of water (a) and gut (b) samples in cRAS and mRAS at Day 50 and Day 72. For each replicate rearing tank (three per RAS) and sampling time there were four individual gut samples and one water sample.

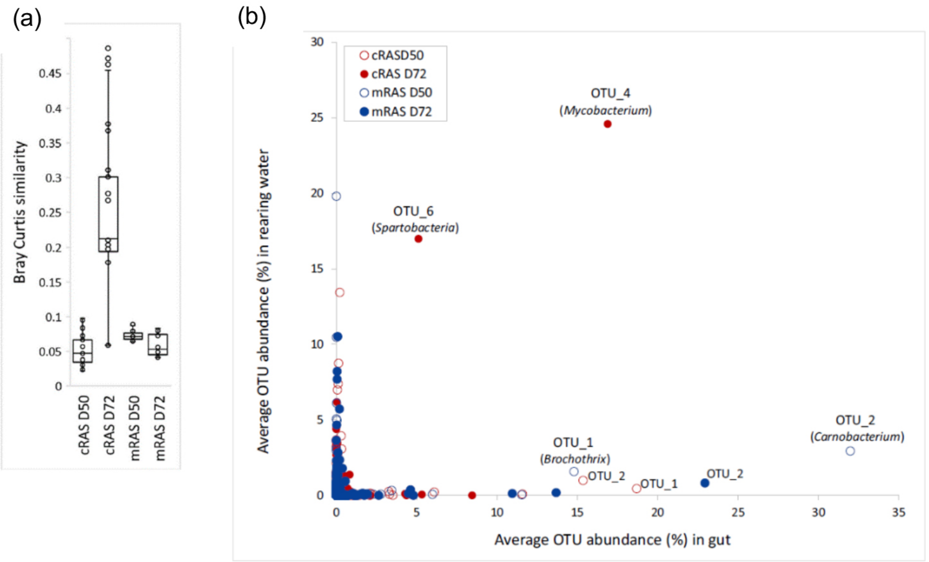

Figure 2. Comparison of rearing water and gut microbiomes. a) Boxplot showing Bray-Curtis similarities between water and gut microbiota. Points represent single comparisons, and the box indicate medians and quartiles. b) Average OTU abundances in gut samples plotted against water samples for all OTUs, in each of the systems and at each sampling time.

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PHYSICAL PLASMA AS AN EFFICIENT AND SUSTAINABLE DISINFECTION TECHNOLOGY IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Aquaculture may contribute to a sustainable and expanding food supply. As this industry has to face problems of cost efficiency (Espinal and Matulić 2019), the reduction of fresh water inflow and the health preservation of the cultivated stock are key factors of imrovement (Yavuzcan et al. 2017). Physical plasma offers a cost efficient method for water disinfection in aquacultural systems and thus represents a leverage for such improvements.

Materials and Methods

Water from a recirculating aquacultural system for the cultivation of *Acipenser oxyrinchus* at the Aquaculture Research Station (Born, Germany) was analysed by ion chromatography to determine especially relevant salts, in addition to pH-value, total organic and inorganic carbon and conductivity. Plasma treatment of this water for 30 min was carried out by an inductively-limited discharge at the water surface for a volume of 500 ml (Schmidt et al. 2019).

Results

Experiments were performed with water from a recirculating aquacultural system (RAS) and also a more reproducible model water, which was composed according to the analytical results. The model water was inoculated with common fish pathogens. Plasma treatment of the water from RAS led to an inactivation of up to 2 orders of magnitude of the microbial load in this particular water. In contrast, the total viable count was reduced by 7 orders of magnitude for *Aeromonas hydrophila* and up to 6 for *Vibrio cholerae*, in the model water. The inactivation was dependent on the incubation time after plasma treatment.

Conclusion

The results confirm the already known antimicrobial efficacy of plasma also for fish pathogens. Organic content is assumed responsible for the less effective inactivation of the microbial load in RAS water. Accordingly, respective processes and technology need to be investigated in more detail.

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MULTI-GENERATION SELECTION AND PERFORMANCE TEST OF AN AFRICAN CATFISH (CLARIAS GARIEPINUS, BURCHELL, 1822) LINE SELECTED FOR BETTER UTILIZATION OF LOW FISH-MEAL FEED

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Introduction

Feed cost is one of the highest expenditures in aquaculture production, mainly due to the provision of sufficient protein-content, especially for carnivorous fish. Nowadays, protein supply is covered from fish meal among others, though fish meal production is gradually decreasing while its price is increasing in parallel. Numerous studies have been performed to investigate different alternative protein sources; however, most of them revealed inadequate growth performance in fish with alternative protein feeds. Nevertheless, better economical indexes could be achieved in many fish species with positive selection for feed utilization. African catfish is an important species in aquaculture production in numerous African, Asian, and European countries. Our aim was to investigate the growth performance of African catfish (*Clarias gariepinus*, Burchell, 1822) stocks with low fish meal feed in a half-industrial experiment and to produce a selected line for better utilization of feeds with alternative protein sources.

Materials and methods

African catfish was selected for better performance fed with low fish-meal feed for two generations and a performance test was performed on the F3 generation. Fish were investigated on a half-industrial scale in a flow-through system using 2 m^3 tanks at the Győri Előre Fisheries Co. site (GYE), with a water temperature of 23-24°C, fed with a control and an experimental diet. The control diet contained 8.1% fish-meal, while the experimental diet contained only 6%. Both feeds with similar pellet size (4.5mm) had the same row protein (42%) and row fat (12%) amount. All generations were produced with 4 multifactorial crosses of 5 male and 5 female individuals. Offspring were reared until reaching the market size and body weight (g) and sex by phenotypic traits was detected. In the F1 and F2 generations (n=1683 and n=1783, respectively) three groups were formed for genetic mapping: two experimental groups in duplicate and one control group. For the production of the F2 generation, the biggest and the smallest specimens of these groups were crossed. Additionally, a positively selected line was created in triplicates by crossing the biggest individuals fed with the experimental feed and by selecting the line several times during breeding. In the F3 generation (n=3083) a control group (C) was created from the group previously fed with the control feed and two experimental groups were created from two of the positively selected groups (PS1, PS2), then all groups were divided into two subgroups and fed with the experimental and the control diet to accomplish a performance test. Data analysis was performed with R software (ver 3.5.3) with $p<0.05$ significance level. Association between feed and sex was investigated with Fisher's exact test, and the effect of feed and sex on body mass was tested with ANOVA analysis using a contrast matrix to keep the first order error low. The F3 groups were tested pairwise with the Welch t-test.

Results

Association between feed and sex could not be detected in any generations. In the F1 generation the groups fed with a low fish-meal diet had significantly lower body mass compared to the control group (control: 1461.6±490.34 g, experimental 1.: 1281.67±515.97 g, experimental 2.: 1076.37±464.36 g). Interestingly, sex had a significant interaction with feed: males had higher body mass with the control diet, while females had higher body mass in the experimental groups. In the F2 generation one experimental group had significantly lower (1389.63±507.22 g), while the other had significantly higher (1827.48±506 g) body mass compared to the control group (1597.05±399.49 g). We could not detect interaction with sex in this generation. A performance test was performed on the F3 generation with a control (C) and two positively selected (PS1, PS2) lines all fed with a control (1) and experimental (2) diet. Both positively selected lines had higher body mass fed with all diets. However, the effect of selection for feed could not be detected since the difference in body mass between different diets was insignificant in all experimental groups (PS1: 1.:1599.11±307.92 g, 2.: 1554.47±315.20 g, PS2: 1.:1542.74±266.84 g, 2.: 1624.48±260.18 g).

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Discussion and conclusion

We can conclude that the lower body mass of the experimental groups fed with low fish-meal diet experienced in the F1 generation is similar to the results found in other fish species. However, in the F2 generation the experimental groups had similar or higher body mass compared to the control group which might be explained by accommodation to the new diet. The effect of the selection for feed could not be detected in the F3 generation, thus the effect of selection for higher body mass was significant, suggesting that there is a good potential for achieving better performance by selection in African catfish.

Acknowledgement

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MOLECULAR ONTOGENY OF THE IMMUNE SYSTEM AND CHARACTERIZATION OF BACTERIAL COMMUNITY STRUCTURE IN FEEDING EUROPEAN EEL (*Anguilla anguilla*) LARVAE

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Introduction

European eel (*Anguilla anguilla*) is a commercially important species for aquaculture in Europe. Commercial aquaculture of this species still depends on the wild-caught juvenile glass eels entering coastal waters, which are farmed until marketable sizes. However, due to historically low levels of recruitment, a supply of hatchery produced glass eels is required to ensure the sustainability of aquaculture and to assist the conservation of this critically endangered species (Pike et al., 2020). Negative host-microbial interactions (Sørensen et al., 2014) and an immune system under development during the early larval stages (Miest et al., 2019), among other factors, have been reported to challenge the European eel larval performance under captive conditions. Feeds are one of the major sources of organic matter supply during first feeding of fish larvae (Vadstein et al., 2018). Food added to the system favours bacterial growth, in response to which fish larvae might develop an immune response. During this experiment, we studied the molecular ontogeny of the immune system of the feeding European eel larvae and characterized the microbial community structure of larvae and the corresponding rearing water throughout feeding culture.

Materials and methods

European eel larvae were obtained through assisted reproduction that has been previously described (Kottmann et al., 2020). When larvae reached the age of nine days post hatch (dph), they were stocked at a density of ~60 larvae L^{-1} in three 8 L "Kreisel" tanks, which were connected to a recirculating aquaculture system. Larvae were fed five times a day with a slurry type diet until they reached 28 dph. Larval survival was monitored daily throughout the rearing period. Larval samples were collected during the endogenous feeding period (on 0 dph & 9 dph) and exogenous feeding period (on 15, 22, and 28 dph) for analysis of the immune and stress related gene expression. Expression levels of 13 target genes, namely complement component C3 (*c3b1*), catalase (*cat*), complement component 1 q subcomponent c chain1 (*c1qc*), immunoglobulin m (*igm*), interleukin 1β (*il1β*), interleukin 10 (*il10*), interferon regulatory factor 3 (*irf3*), interferon regulatory factor 7 (*irf7*), major histocompatibility complex class ii (*mhc 2*), toll like receptor 18 (*tlr18*), tumor necrosis factor β (*tnfb*), heat shock protein 90 (*hsp90*) and heat shock protein 70 (*hsp70*) and 3 reference genes, namely elongation factor 1 (*ef1*), 18s ribosomal RNA (*rsp18*), and tubulin β (*tubb*) were determined by quantitative real-time PCR. Relative expression of targeted genes was calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). For bacterial community composition analysis, larval samples and samples of rearing water were collected at each sampling point (on 0, 9, 15, 22 and 28 dph). 16S ribosomal RNA gene (rDNA) amplicon analysis is currently being carried out to characterize the bacterial community composition in larval and water samples.

Results and discussion

Expression of all immune and stress related genes studied were significantly ($P<0.05$) influenced by larval age, except the *il10* gene, which showed a constant activity throughout ontogeny, probably indicating basal immuno-protection. Analysis of the first results indicates three further expression patterns. Genes, *c3b1* and *igm* showed highest expression levels at hatch (0 dph) and expression levels decreased progressively with age, probably indicating the maternal origin of these genes, which most likely provided initial protection to the newly hatched larvae (Miest et al., 2019) (Figure 1 A). Moreover, *il1β*, *tnfb*, *irf7* and *hsp90* were expressed at lowest levels on 0 dph and increased with age, providing means of progressive increase of the larvae's immuno-protection. Interestingly, the expression pattern of stress related *hsp90* showed a significant peak on 22 dph, indicating a period of higher activity of the stress/repair mechanism. This, probably signals the effects of the point of no return (around 20-24 dph), resulting in a selection of those larvae that timely and successfully initiated first-feeding (Figure 1 B). Furthermore, *c1cq* and *irf3* showed an upregulated expression on 9 dph, when larvae gained feeding functionality, while expression levels of these genes remained at relatively high levels, indicating the own immune-readiness of larvae throughout the first-feeding and feeding window (Figure 1 C). More genes are being analysed to understand their characteristic expression patterns. Bacterial community composition analysis is also currently being carried out for larval and water samples collected during the experiment.

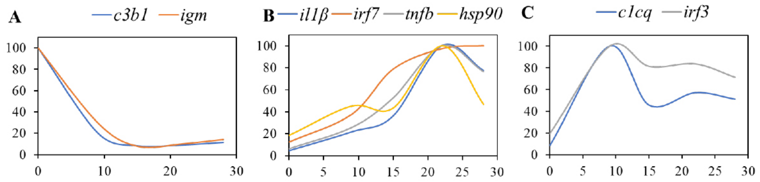

Figure 1: Expression patterns of immune and stress related genes. The x-axis displays age (in days post hatch), and the y-axis displays relative gene expression.

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MONITORING THE SPATIAL DISTRIBUTION OF FARMED FISH IN LARGE COMMERCIAL SEA CAGES DEPLOYING A MULTI-BEAM SONAR

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Introduction

Spatial distribution of Atlantic salmon (*Salmo salar*) in sea cages is an important welfare indicator. Uneven distribution of salmon inside the sea cages can be associated with an avoidance of unfavorable environmental conditions such as low oxygen, strong light, and suboptimal water temperature (Oppedal et al. 2011). With a good understanding of how salmon are distributed in a sea cage under preferable environmental conditions, we can detect unfavorable environmental conditions just by monitoring changes in their spatial distribution patterns. Echosounders have been used to investigate vertical fish distribution patterns in sea cages (e.g. Bui et al. 2013). These studies brought valuable insight into fish vertical distribution patterns in relation to environmental factors. In Norway, large sea cages can hold up to two hundred thousand individuals. It is likely that salmon distribution patterns inside such large structures are uneven both horizontally and vertically. Single beam echosounders can monitor only a small proportion of the sea cage volume, thus a more reliable assessment of salmon distribution patterns in the entire cage is required. In this study, we use a novel multi-beam sonar WASSP (ENL Group, A Furuno Company, New Zealand) to obtain more representative information of salmon distribution patterns in large commercial sea cages. The sonar can perform 120° fan-shaped scanning, which allows to scan larger proportions of the sea cage volume. Our long-term goal is to understand salmon distribution patterns in large sea cages in relation to environmental and fish-internal factors. Spatial distribution patterns of salmon can be used as behavior-based welfare indicators, which will enable more efficient farm operations (e.g. behavior based feeding, avoidance of stressful operation when fish show unusual behavior). As a first step, we conducted pilot study using WASSP, to establish the method to properly monitor salmon spatial distribution patterns in large commercial sea cages.

Materials and methods

The pilot study was carried out at a commercial Atlantic salmon farm located in Møre and Romsdal county in Norway. We collected data at 2 different sea cages which contained approx. 200,000 (average weight approx. 400g) and 70,000 salmon (average weight approx. 2kg), respectively. The sonar was mounted on a rig with a fixed 30° tilt so that the coverage of the sea cage volume by the sonar was maximized. The rig was placed inside the cage close to the cage net, and it was manually rotated following a horizontal angle of approx. 120° to scan more than 80% of the cage volume. We collected data using combinations of different acquisition ranges (25 m, 40 m, and 70 m) and rotation speeds (6 s/120° and 12 s/120°) of the sonar. 9 GoPro cameras were placed in the sea cage for simultaneous visual monitoring and validation. 8 cameras took images at a frequency of 1 Hz to get a rough estimation of fish density at different locations and depths at a time. A video was recorded next to the sonar rig to assess the potential influence of the rotating rig on salmon behavior (see Fig. 1 for experimental setup). The sonar data is post-processed in Echoview (Echoview Software Pty Ltd., Australia) to filter out all the noises (i.e. echo signals from anything other than salmon in the sea cage) and to create 3D distribution maps of salmon. Relevant environmental data (dissolved oxygen, temperature, salinity, feeding activity) were collected during the data collection with the sonar.

Results

Preliminary results showed that the multi-beam sonar monitors salmon spatial distribution inside sea cages regardless of the fish size. Difference in distribution was evident between small and large fish. Camera data showed that large fish avoided the shallow water layer down to around 8 m, whereas small individuals were present from around 4 m. Sonar data agreed with these findings. The sonar was capable of shooting through the thick layer of fish and detecting individuals distant from the instrument. We are currently post-processing the sonar data to assess the effects of different acquisition ranges, rotation speeds, and the fish size on the sonar data quality, as well as the distribution patterns of salmon. The work we will present is a part of our ongoing research effort.

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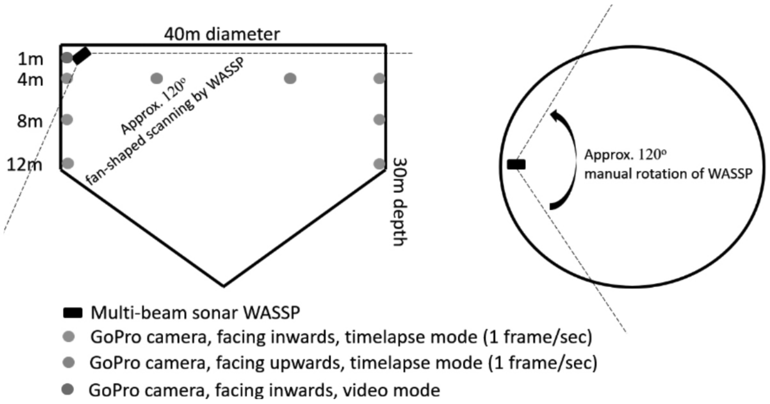

Fig. 1. Illustration of camera deployment and placement and rotation of the multi-beam sonar WASSP. Left: side view of the sea cage. Right: top view of the sea cage.

Discussion

This study shows that the multi-beam sonars have a great potential as a tool to properly monitor salmon distribution patterns in large commercial sea cages. To the best of our knowledge, multi-beam sonars have rarely been used to monitor the salmon distribution in the sea cages, which may be due to the complexity and the enormous size of the data which derives from the instruments. With the use of multi-beam sonar, we can provide more representative information of salmon spatial distribution patterns in large sea cages. We are aware of the potential bias in the sonar data due to shadowing effects by the dense layer of fish. However, it is already shown that WASSP can overcome this challenge. In the future, the rotation of the sonar can be automated so that we can continuously and autonomously monitor salmon distribution patterns and better understand how they react to external and fish-internal factors. From a fish welfare perspective, the use of hydroacoustic monitoring methods such as multi-beam sonar is preferred as it does not require direct handling of fish and applies little stress on fish. Therefore, it is critical to make a continuous effort to apply and refine hydroacoustic tools for the assessment of fish welfare.

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HISTOLOGICAL CHANGES IN GILTHEAD SEABREAM (*Sparus aurata*) GILLS AFTER EXPOSURE TO CLOVE OIL COMBINED WITH LIDOCAINE

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Introduction

Animal welfare has become a relevant topic to minimize pain during procedures. Anaesthesia is commonly used in aquaculture and research, but it is necessary to choose the best anaesthetic and the ideal concentration to avoid side effects. Clove oil is a natural anaesthetic alternative to synthetic compounds like MS-222 and 2-phenoxyethanol. Another emerging issue is the use of analgesics, which may improve fish well-being and have synergy effects when anaesthetics are used. This study aims to observe the effect of clove oil (an anaesthetic) and lidocaine (an analgesic) on histological changes in gilthead seabream (*Sparus aurata*) gills.

Materials and Methods

This study was carried out in CETEMARES - Marine and Environmental Sciences Center MARE-Polytechnic of Leiria, Peniche with seabream (61.61 ± 14.57 g). A refinement pre-trial study was performed to determine the best anaesthesia concentration to use. For the trial, an anaesthetic, clove oil (45 mg/L) and an analgesic, lidocaine (2.5 mg/L) were used. With these compounds, two treatments were performed: clove oil (CO) and clove oil + lidocaine (CO + L). The CO + L fish, were first exposed 5 minutes to the analgesic and then set to anaesthesia. Each fish was individually anaesthetised until they reached the deep anaesthesia stage (A5). Then, each fish was transferred to an overdose of the anaesthetic. In each of the treatments (n=10) the gills were sampled for histological analysis. They were fixed in 10% formalin for 24 hours and then transferred to 70% ethanol until processing. Standard histology techniques were used, and sections (5 µm) were stained with Haematoxylin-Eosin. For gill observation, we used, Ortiz-Delgado et al. (2007) protocol, using an optical microscope, Leica DM2000 LED, and the images were captured using a digital camera, Leica MC 170 HD. The percentage obtain corresponds to the number of fish where gill histological changes were observed.

Results and Discussion

The gills are an organ with a large surface of contact with the external environment, through the epithelium, which facilitates monitoring the influence of the aquatic environment on living beings (Marinović et al., 2021).

Through gill histology, it was possible to observe changes in structures (epithelium rupture and lamellar fusion) and morphology (circulatory disorders, lamellar hyperplasia, hypertrophy and hyperplasia from chloride cells) when the anaesthetic was administered, with or without analgesic.

Epithelium rupture was higher when lidocaine was used (80%) comparing with CO (40%). This being the consequence of the addition of one more chemical, causing damage to the gill epithelium, not only because of the concentration but also by a longer exposure time (Reddy & Rawat, 2013).

Chloride cells play a role in ionic transport with the possibility of being involved in detoxification (Mokhtar, 2017). With lidocaine (CO + L) hypertrophy of chloride cells was higher (80%) comparing with single use of CO (50%) probably due to the exposure of another chemical component (Macirella & Brunelli, 2017). Chloride cell hypertrophy can be identified as an adaptive response due to their ionic function to balance with the external environment (Strzyżewska-Worotyńska et al., 2017).

Conclusion

In this study, the use of clove oil as an anaesthetic, and lidocaine as an analgesic showed histological changes in *S. aurata* gills. More tissue changes were presented when using the combination of compounds, due to the increased use of chemical compounds, inducing higher histological changes during exposure.

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NUTRITIONAL VALUE AND QUALITY CHANGES DURING FROZEN STORAGE OF ECO-INNOVATIVE BIOFORTIFIED FARMED GILTHEAD SEABREAM (*Sparus aurata*) AND COMMON CARP (*Cyprinus carpio*)

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Introduction

Farmed gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) are two of the most valuable commercial fish species produced in Europe, representing, respectively, 16% and 11% of the European aquaculture production and 9% and 7% of the European total apparent consumption (EUMOFA 2020). One third of the world’s population has severe nutritional deficiencies, particularly of iodine (I), selenium (Se), and iron (Fe), which can create adverse effects on human health (Allen et al., 2006). The regular consumption of fish can make an important contribution to make up for the lack of nutrients and in particular to referred micronutrients. Farmed fish is a key resource to meet the increasing seafood demand due to its potential to develop tailor-made biofortified fish products addressing consumers’ nutritional requirements through sustainable, natural, safe, and high-quality feeds (Allen et al., 2006, FAO, 2020). Additionally, consumers’ demand towards easy-to-prepare, ready-to-eat and ready-to-cook food products with extended shelf-life is gaining importance, with frozen products representing 35% of fish and fish products trade (FAO, 2020). The present study aimed to evaluate the effects of frozen storage during 12 months on nutritional values and quality changes of farmed gilthead seabream and common carp biofortified with iodine-rich seaweed (*L. digitata*) and selenium-rich yeast as feed ingredients.

Material and methods

Two diets, (1 control and 1 biofortified with I-rich seaweed and Se-rich yeast) were tested for 3 months in each fish species, simulating a finishing diet period. At the end of the trials, fish were slaughtered following the typical commercial practices. Freezing, glazing and frozen storage were performed according to conventional industrial process, and fillets were collected without skin whenever required. For each treatment, control (CTR) and biofortified (BF), glazed fish fillets (gilthead seabream) or whole fish specimens (common carp) were randomly packed in polyethylene terephthalate (PET) bags and stored in cartoon packages at –20 °C in a frozen food storage chamber over 12 months. Samples ($n = 6$ fish fillets) were taken before (day 0) and after 45, 135, 225 and 360 days of frozen storage. Frozen samples were thawed under refrigeration (4 ± 1 °C) for approximately 12h, and fish fillets were divided in sections for macro, trace and toxic elements content, lipid oxidation (LPO), water holding capacity (WHC), colour and texture analyses.

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Results and Conclusions

Results showed that, overall, biofortification strategies through dietary supplementation with I-rich seaweed (*L. digitata*) and Se-rich yeast in gilthead seabream and common carp did not significantly affect the quality parameters during frozen storage. Generally, elements content in seabream fillets were stable, being only found some fluctuations, namely the decreased I content in BF fillets after 135 days of storage, Br content after 45 days and Fe content after 22 days of storage. In general, BF fillets revealed significantly higher contents of I and Se during frozen storage, compared to CTR fillets. Similarly, no specific trend was observed in carp fillets during frozen storage, being only found some fluctuations, namely higher Fe content in BF fillets at days 45 and 360, Ca after 360 days and Cl after 45 days, as well as lower content of As after 135 days of storage. Common carp BF fillets revealed significantly higher contents of I, Se, Zn, Br and As throughout the storage period, compared to CTR fillets. Frozen storage resulted in decreased WHC and increased lipid oxidation (TBARs) values in both BF and CTR gilthead seabream and common carp fillets during 360 days of storage. In terms of colour attributes, no specific trend was observed in both BF and CTR gilthead seabream and common carp fillets. Yet, increased redness and decreased yellowness were observed in BF and CTR gilthead seabream fillets after 45 days of storage, resulting in increased colour intensity. On the other hand, higher lightness and whiteness values were registered in BF common carp fillets, as well as in CTR fillets after 225 days and 45 days of storage, respectively. Overall, the industrial frozen storage did not compromise the nutritional quality of the tested BF gilthead seabream and common carp, particularly in terms of TBARs, WHC, texture and colour, with glazed fillets maintaining good quality during the 360 days of storage at –20 °C.

Acknowledgements

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INFLUENCE OF *Microchloropsis gaditana* EXTRACT SUPPLEMENTATION ON THE GROWTH, MORPHOLOGICAL, AND BODY QUALITY TRAITS OF ASIAN SEABASS *Lates calcarifer* AT TWO DIFFERENT SALINITIES

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Introduction

Asian seabass, *Lates calcarifer* is a well-known farmed species with an established market. It is a warm water carnivorous, fast growing euryhaline fish (Schipp et al, 2007). These biological traits together with its consumer acceptance points to the potential for expansion of barramundi farming worldwide (Lawley, 2010). The cultivation of this species in RAS (recirculating aquaculture system) needs further development (Larkin, 2000) and the present study compare the growth performances and morphological – body quality traits of Asian seabass at two different salinity levels, testing also a microalgae extract (*Nannochloropsis gaditana*) for potential benefits to fish growth, health, and fillet traits (Nagappan et al, 2021).

Material and methods

Asian seabass, *Lates calcarifer* (50 fish/tank; initial weight of 64.9 ± 0.1g) reared at two different salinities, 38+/-2ppm (S1) and 14 +/- 2ppm (S2) in RAS. Fish in both systems were fed four times daily to satiation with either a control diet (CTRL) or the CTRL diet supplemented with *N. gaditana* liquid extract top coated at 3% (W/V) (ALG). After the 10 week trial period, a bulk sample weight of all tanks was performed and 8 fish from each tank were sampled to carry out carcass analysis, obtain biometric-morphometric data, as record body quality traits.

Results

At the end of feeding trial, no significant differences were found in the average final weight and SGR among the different treatments. Both treatments reared in S1 had significantly higher FCRs than the FCRs of both treatments reared in S2, compared whilst feed intake of fish in S2 were significantly higher than that of fish fed CTRL (S2)

Although numerical differences were apparent, all biometric, morphometric indexes and fillet yield and quality parameters except VSI were found not to be statistically different between treatments. VSI was significantly higher in fish from S1 compared to S2. Results of body colour intensity (L*, a* and b* coordinate) using a Minolta Chroma-Meters CR-400 cat. 1878-209 showed no significant difference between S1 and S2 in both treatments.

	CTRL (S1)	ALG (S1)	CTRL (S2)	ALG (S2)
Initial weight (g)	64.9 ± 0.12	64.9 ± 0.08	64.9 ± 0.15	64.9 ± 0.12
Final weight (g)	272.3 ± 9.21	268.51 ± 9.28	257.83 ± 7.57	263.19 ± 4.75
FCR	0.93^{a} ± 0.02	0.94^{a} ± 0.01	0.89^{b} ± 0.01	0.90^{b} ± 0.01
SGR (%/day)	2.02 ± 0.05	2.00 ± 0.05	1.94 ± 0.04	1.97 ± 0.02
Feed intake (kg)	9.62^{a} ± 0.25	9.53^{a} ± 0.47	8.59^{b} ± 0.28	8.94^{ab} ± 0.13
SFR (% of total biomass)	1.81 ± 0.01	1.80 ± 0.03	2.11 ± 0.72	1.72 ± 0.02
K-factor	1.46 ± 0.08	1.43 ± 0.06	1.34 ± 0.04	1.34 ± 0.07
Fillet Yield %	43.26 ± 0.95	44.58 ± 0.18	45.37 ± 2.12	43.42 ± 2.07
VSI	10.19^{a} ± 0.82	10.14^{a} ± 0.48	8.92^{b} ± 0.42	8.98^{b} ± 0.47
HSI	1.91 ± 0.24	2.07 ± 0.18	1.78 ± 0.10	1.62 ± 0.11
SSI	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
L*	42.58 ± 0.74	42.51 ± 0.92	42.30 ± 1.59	42.87 ± 2.25
a*	0.19 ± 0.41	0.08 ± 0.17	0.19 ± 0.84	0.43 ± 0.50
b*	3.77 ± 0.95	4.12 ± 0.78	3.33 ± 0.83	3.90 ± 1.00

Values in the same row with the different superscript letters are significantly different ($P < 0.05$).

Table 1 Growth performance of Asian seabass after 10 weeks feeding on experimental diets (mean ± standard deviation) (CTRL, control; ALG, test diet; L*, lightness; a*, red/green coordinate; b*, yellow/blue coordinate.)

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THE EFFECT OF FLOWRATE IN RECIRCULATING AQUACULTURE SYSTEMS ON WATER CHEMISTRY AS IT CONCERNS FEEDING EVENTS OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

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A series of investigations were undertaken related to inlet configuration and flow rate on efficiency of solids removal, maintenance of water quality, and production and welfare indicators in farmed Rainbow trout (*Oncorhynchus mykiss*) in Recirculating Aquaculture Systems (RAS).

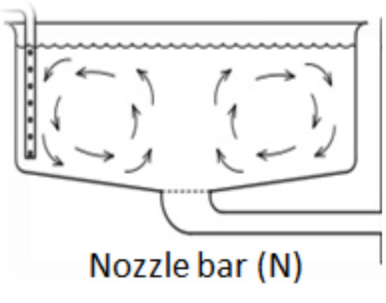
To assess solids removal efficiency of uneaten feed particles during feeding in circular RAS tanks, two inlet design placements (nozzle bar and elbow pipe), and three flowrates were tested including inlet pipe with multiple holes ensuring water input at varying depths (Image 1), and elbow pipe (Image 2) where the angle of inlet was adjusted for water to move parallel to the tank wall (Image 3). Flow rates of 600L/h, 800L/h (normal for trout juveniles), and 1000L/h were investigated, totalling 6 treatments with a combination of variables, inlet type, and flow rate.

In unstocked tanks, feed pellets were distributed at the surface as per normal feeding practice and their movement recorded on overhead video cameras. Primary and secondary flow were assessed, as well as pellet distribution in the tank, settling location at the tank bottom and number of pellets entering the central drain. Primary flow was observed in all treatments and tanks with higher flowrate displayed stronger primary flow in both inlet configurations. Primary flow in the elbow configuration decreased with increasing depth regardless of flow rate. Higher flowrate created stronger secondary flow patterns and the nozzle bar configuration produced higher secondary flow than the elbow pipe, thus, pellets settled earlier than in elbow configured tanks with a nozzle inlet bar. Use of the nozzle bar inlet configuration with higher flowrate showed the most optimal option for waste removal of uneaten feed particles in tested circular RAS tanks.

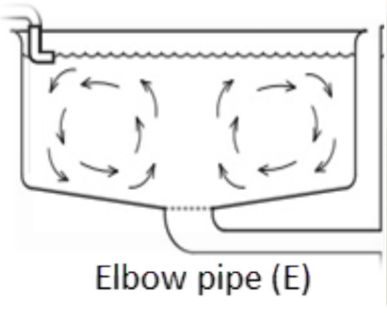
Efficiency of the six treatments for reoxygenation of the system was tested in unstocked tanks by using an oxygen scavenger (Na_2SO_3) to deplete the isolated tanks oxygen level to ~0% and then restoring water flow to assess time needed for each configuration to restore to 100% saturation. At lower flow rates (600L/h) there was a lower reoxygenation rate using the nozzle bar compared with the elbow pipe (N=2), but at higher flow rates the two configurations did not vary significantly and at 1000L/h could achieve tank reoxygenation rates of 1.9 - 3%/min regardless of configuration.

In replicate (3) tanks stocked with 9±0.35g rainbow trout at optimal production conditions (16.5 °C, 1-2ppt salinity, > 6.5 mg/l DO, with automated O_2 injection) considering only the more efficient nozzle bar configuration at the same 3 flow rates (600L/h, 800L/h, 1000L/h), variation in oxygen and CO_2 level were recorded during feeding at 4 samplings over 6 weeks of fish growth. Fish were initially fed at 3% body weight/day, gradually reduced to 2.5%. Feeding was performed by hand 3 times per day at 9.00, 12.00 and 15.00.

1)



2)



3)

(Continued on next page)

Changes to oxygen and CO_2 levels were recorded at 5-minute intervals with a handheld probe from 5 minutes prior to feeding to 1 hour after feeding commenced in the tanks and at the inlet supply. To calculate oxygen consumption during feeding, only the negative data slope was analysed, together with the constant oxygen supply of the system. The oxygen consumption of each treatment during feeding increased significantly as the fish grew and stocking density increased ($P=0.000$), but no difference ($P > 0.05$) was measured within the flow rate treatments in any of the sampling weeks. To calculate CO_2 increase, only the positive data slope was analysed. The carbon dioxide generation changed significantly as fish size, and thus stocking density, increased ($P=0.002$) but no difference was measured among the flow rate treatments in any of the sampling weeks.

Oxygen level was also monitored continually (every 10 mins for 6 weeks) in tanks to detect patterns associated with fish handling events on experimental sampling days. This data showed greater variability in oxygen level, indicating metabolism related oxygen drops and system O_2 injection on days of sampling events compared to non-sampling days, when fish disturbance was only caused by regular husbandry. Additionally, tanks with higher flow rates were shown to mitigate these effects better than tanks with lower flow rates, demonstrating that these higher flow rates assist in homogenising the water environment for fish, and can smooth the effects of handling events on O_2 fluctuations.

Growth performance was assessed for fish in all treatments throughout the experimental period including final weight, FCR, SGR, visual indicators of welfare, as well as condition factor (K) and visceral fat content. Flow rate was not found to have any effect on these factors.

Nozzle-bar inlet placement benefits waste removal performance and best homogenises water quality. Juvenile trout were not negatively affected by higher flowrates regarding either growth performance or welfare, but this is an interesting field of further investigation. Higher flowrates are considered to have better system efficiency in RAS in terms of waste removal and tank reoxygenation rate, further mitigating O_2 stress of fish during growth. Additional studies must establish upper biological limits for fish welfare while considering other interacting factors of RAS.

CONTACT PLATES DESIGNED FOR PRACTICAL USE IN HATCHERIES: POST-LARVAE AND SPAT SAMPLES

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Introduction

Nowadays, hatcheries are essential to sustain bivalve aquaculture. In addition to the larval stage, these facilities must maintain the individuals in culture until they reach the appropriate size to be taken to the outside environment. From the time of attachment and metamorphosis, the specimens are handled as little as possible. The culture usually takes place in bins with forced flow or, in species such as razor clams, buried in sand. During this phase there are episodes of mortalities that should be investigated, since they represent an important economic loss, considering the advanced stage of the culture.

Sampling for microbiological control in hatcheries should include simple procedures that are easy to use by personnel, without the need for complex specific training. In addition, they should be fast processes, given the volume of work in these facilities during peak production periods.

Materials and methods

The USC team developed contact plates, of their own design, based on Marine Agar and Thiosulphate-Citrate-Bile-Sucrose (TCBS) media, commonly used in microbiological controls in the hatchery. The objective was to determine their usefulness for direct sampling of bivalve seed and to evaluate their ability to detect bacterial pathogens.

The Centro de Cultivos Mariños carried out tests with specimens of *Venerupis corrugata* (pulled carpet clam), *Ruditapes decussatu*s (grooved carpet shell) and *Ensis magnus* (razor clam). The samples were taken according to production requirements and routine protocols, comprising sizes between 180*µ* and 6 mm.

Further processing was carried out by the USC-team. Bacterial isolation, preservation and identification of isolates followed the methodologies described in Prado et al. (2014).

Results and discussion

The plates proved to be suitable for processing the individuals, regardless of size, within the tested range.

The presence of known bacterial pathogens in aquaculture could be detected in the processed samples, such as *Vibrio splendidus*-like (VSL), *V. tapetis*, *V. ostreicida*, *V. alginolyticus*, *V. europaeus*, *V. mediterranei*.

The results demonstrate that the designed contact plates are suitable for simple and rapid sampling in the hatchery, with minimal handling of the individuals, to obtain useful results that allow the identification of marine aquaculture bacterial pathogens.

They therefore fulfil one of the objectives of their design, although it should be noted that the post-processing of the samples obtained can be somewhat more complex than that of standard plates and must be carried out by specialised personnel.

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Acknowledgements

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STUDY OF *Venerupis corrugata* MORTALITIES IN A RAFT CULTURE EXPERIMENT AT DIFFERENT DENSITIES

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Introduction

Bivalve mollusc aquaculture currently needs the supply of hatchery-produced seed to sustain its activity. In addition to larval culture, it is necessary to address pre-fattening, in facilities on land with forced flow or in floating structures ("bateas") with passive flow.

The IGaFA has a mini-hatchery facility with a light greenhouse-type structure for the cultivation of seed of molluscs of commercial interest in Galicia (NW Spain). In this work, we report the results of a pre-fattening trial of pulled carpet clam, *Venerupis corrugata*, in raft at different densities are shown. During development, mortalities were recorded that do not seem to be justified by the densities tested. Given the importance of bacterial pathogens in bivalve mortalities, in larval culture, but also in an external environment, a microbiological study was carried out to evaluate the possible role of bacterial pathogens in the losses suffered.

Materials and methods

At the facilities of the IGaFA, belonging to the Consellería do Mar-Xunta de Galicia, located in Illa de Arousa (Pontevedra, Spain), the hatchery culture of the species *Venerupis corrugata* (Gmelin, 1791) was carried out up to the seed stage.

Two batches of reproductive specimens from the natural bank of "O Bohido" in the Illa de Arousa, which were induced to spawn by thermal shock, were used as the starting point (November 2021). Both batches were developed in the hatchery and the nursery, following the routine procedures, until March 2022, when they were transferred to the outside environment, to a "batea" (raft) in the sea.

On 30/06/22 an experience of seed culture was started with both batches. Cylindrical lanterns with a diameter of 40 cm and 2 mm mesh were used. To determine the possible culture results, using different densities, a trial was designed in which a total of 9 lanterns were used to test 3 different densities (2.000 individuals per floor, 1.000 individuals per floor and 500 individuals per floor). In each lantern, three floors were arranged at the same test density, with floor 1 being the shallowest part of the water column and floor 3 the deepest. The maximum density tested of 2.000 individuals per floor corresponds to 1.591 indiv/cm^2, and the following ones to 0.795 indiv/cm^2, and 0.397 indiv/cm^2, respectively.

On August, the mortality rates in each compartment were recorded. Bacteriological samples were taken and processed in situ, using contact plates designed by USC-team, with Marine Agar (MA) and Tiosulphate-Citrate-Bile-Sucrose. Further processing is carried out by the USC-team. Bacterial isolation, preservation and identification of isolates followed the methodologies described in Prado et al. (2014).

Table 1. Mortality rates in the lanterns at the different densities assayed (high, medium, low) and on the floors within each (upper, middle, lower) recorded after one month.

DENSITY	FLOOR		
	Upper	Middle	Lower
High	0	17.50	46.15
Medium	45.20	58.40	21.60
Low	26.20	32.20	52.20

(Continued on next page)

Results

The sampling carried out on August showed the mortality rates detailed in Table 1.

On the basis of the preliminary mortality data recorded, it does not appear that density can be responsible for the deleterious effects detected.

Preliminary results of microbiological analyses recorded a high presence of vibrios in the samples, including pathogens: *Vibrio ostreicida*, *V. alginolyticus-V. parahaemolyticus*, *V. mediterranei*. Their distribution pattern, as well as the presence of other taxa, is being studied to assess a possible role in the recorded mortalities.

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TEMPERATURE RESTORES MUCOSAL BARRIER INTEGRITY AFTER A FISH CUTANEOUS INFECTION

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Recent findings from our lab suggest that, contrary to the long-standing paradigm, fish do not have an under-developed immune system compared to mammals. However, because fish lack endogenous metabolic-driven endothermy, they require movement to/from warmer temperatures at different stages of disease to activate protective antimicrobial mechanisms. These dynamic temperature changes are at the core of the crosstalk between fish, pathogens and the environment. Thermal promotion of immune competence is achieved through a combination of earlier, tailored defenses that are quickly followed by efficient inflammation control. This allows for both enhanced antimicrobial protection as well as efficient use of energy resources. Together, this represents an important shift in our understanding of fish immunity and has major applied implications for the aquaculture sector. This presentation will describe salient contributions of thermoregulation during an *Aeromonas* infection, with a focus on restoration of barrier integrity following cutaneous tissue damage.

DIETARY ADDITIVES SUPPLEMENTED IN INERT MICRODIETS CAN MODULATE THE EXPRESSION OF IMMUNITY RELATED GENES OF WHITELEG SHRIMP (*Penaeus vannamei*) POST-LARVAE

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Introduction

The whiteleg shrimp (*Penaeus vannamei*) is currently the second most representative animal species in aquaculture, constituting in 2018 a share of 6% in global production. To meet the market demands, whiteleg shrimp larvae and post-larvae (PL) yields in hatcheries increased intensively, globally originating around five million tonnes of adult individuals per year. Nevertheless, initial developmental stages are critical and frequently associated with sub-optimal growth and low survivals, which, in some cases, can be related with nutritional deficiencies or infections by opportunistic pathogens. Hence, problems in larviculture can have an enormous impact on shrimp performance in the long-term, affecting the downstream production of high-quality juveniles and adults. In fact, industrial shrimp farming is characterized by inconsistent yields as it is extremely susceptible to severe pathogenic episodes that result in disastrous consequences to production and significant economic losses (Kibenge, 2019; Shinn et al., 2018; Zou et al., 2020). Besides the fact that shrimp cannot be vaccinated, the use of antibiotics in the aquaculture industry is limited due to inherent food safety concerns, environmental issues, and the increased antimicrobial resistance (Lulijwa et al., 2020). Consequently, the use of functional dietary additives to stimulate the shrimp immune system has been studied as a prophylactic alternative and is regarded as an extremely important strategy to overcome the constrains of intensive shrimp farming. However, information is scarce regarding the effects of using dietary additives for the shrimp early life stages of development and how it could potentially benefit whiteleg shrimp larviculture. Therefore, this study aimed to evaluate the effects of several health promoting nutrients and additives (i.e. vitamins C and E, beta-glucans, taurine and methionine) supplemented in microdiets on the growth performance, oxidative status, immune condition and gene expression of whiteleg shrimp post-larvae.

Methods

Four experimental microdiets were evaluated in triplicates. A commercial like diet was used as positive control (PC), whereas three experimental diets were formulated based on the PC diet. A negative control diet (NC) presented lower supplementation levels of vitamin C and E; the PC diet supplemented with taurine plus methionine (T+M); and the PC diet supplemented with β-glucans (BG). Whiteleg shrimp post-larvae (mean wet weight 9 mg) were kept at around 28 ºC and fed *ad libitum* for 18 days. At the end of the trial, shrimp were weighted for growth performance determination and samples were collected to assess oxidative status, immune condition and gene expression.

Results and discussion

Increasing the Vitamin C and E supplementation levels in the PC treatment did not produce any changes in growth performance, survival, immune and antioxidant parameters when compared to the NC diet. However, the relative expression of the *pen-3* gene in shrimp fed the NC diet was lower than in those fed the PC diet, suggesting that increasing the levels of these vitamins may improve the shrimp resistance to pathogenic agents, as penaeidins have antibacterial and antifungal activities.

PL fed with the T+M diet showed similar growth performances, survival, oxidative status and immune condition to those fed the PC diet but showed significantly higher relative expressions of the gene *crus*, and significantly lower relative expressions of the gene *hmc*, when compared to those fed the NC diet. On one hand, the supplementation of taurine and methionine seems to compensate the decrease of vitamin C and E levels in the diet and increase the shrimp immune response through higher levels of *crus* expression, but on the other hand, the expression of the *hmc* gene decreased.

The inclusion of β-glucans in the diets did not affect the PL growth performance and survival. Nonetheless, lipid peroxidation levels were significantly lower when compared to those fed the PC diet, suggesting that β-glucans conferred antioxidant properties to the diet. When compared to the NC diet, PL fed with BG showed significant increases in tGSH activity levels and in the relative expression of genes *crus* and *pen-3*, suggesting that the β-glucans supplementation can boost the antioxidant capacity and immune condition of whiteleg shrimp PL when the levels of vitamins C and E are reduced.

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In conclusion, the results obtained in this study demonstrate that although no improvements in growth performance and survival were observed, all dietary additives tested have the potential to add value to inert microdiets for whiteleg shrimp PL. Amongst the additives tested, the inclusion of β-glucans in the diets seems to be the most promising one as it reduced lipid peroxidation in the shrimp when compared to the commercial-like diet.

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PROBING THE PROPHYLACTIC POTENTIAL OF GINGER EXTRACT AGAINST ACUTE HEPATOPANCREATIC NECROSIS DISEASE-CAUSING *Vibrio parahaemolyticus* USING GNOTOBIOTIC *Artemia franciscana* AS A MODEL ORGANISM

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Introduction

The annual production of crustaceans has recorded over 9.4 million tons, which is valued at about US$ 69.3 billion (FAO, 2020). However, this growing industry, which is dominated by penaeid shrimps is significantly impacted by disease outbreaks. Acute hepatopancreatic necrosis disease (AHPND) is one of them caused by *Vibrio parahaemolyticus*. To prevent the occurrence of AHPND in shrimps, there has been an increasing effort in developing anti-infective strategies. In this context, nutraceuticals derived from plant sources (e.g. ginger) are being increasingly studied for their disease control biological potential, with the aim to use them as anti-AHPND agents as an alternative to unsustainable chemotherapeutics, such as antibiotics. The plant-based nutraceuticals are non-toxic, biodegradable, and biocompatible (Citarasu, 2010). In the present study, gnotobiotic *Artemia* was used as a biological model to determine the prophylactic potential of ginger extract (GE) against AHPND-causing *V. parahaemolyticus*.

Materials and Methods

At first, a toxicity assay was performed in which *Artemia* were pretreated with increasing concentrations (84, 125, 167, 208, 250, 333 ug/ml) of GE. Non-pretreated *Artemia* were maintained as a control. Secondly, an *Artemia* challenge test was performed using selected non-toxic doses of GE, and as a readout, the survival of *Artemia* challenged with *V. parahemolyticus* was recorded. The dose that showed the most prominent anti-AHPND effect was selected to study the mode of action of GE by focusing on the host *Artemia* immune responses, which include heat shock protein, prophenoloxidase, transglutaminase, peroxinectin, and superoxidase dismutase using qPCR assay. The toxicity and challenge assays were performed in quintuplicate.

Results

The results showed that pretreatment of *Artemia* larvae with GE at a dose of 250 ug/ml led to significant protection of the larvae upon challenge with AHPND-causing *V. parahemolyticus*. Our results also showed that the increase in the survival of the pretreated larvae was associated with a significant increase in the expression of a core set of genes associated with the defense system of *Artemia* i.e. heat shock protein 90, lipopolysaccharide and β-1,3-glucan-binding protein, prophenoloxidase, peroxinectin, superoxide dismutase, transglutaminase1. From the overall results, it can be concluded that ginger extract could strongly enhance the resistance of the host *Artemia* against *V. parahaemolyticus* and thus can be used as a potentially effective prophylactic agent for controlling AHPND in farmed shrimps.

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DEFATTED *Tenebrio molitor* LARVAE MEAL AS PARTIAL AND TOTAL FISHMEAL SUBSTITUTE IN DIETS FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*): IMPACT ON SHORT- AND MID-TERM HOMEOSTATIC REGULATION OF FOOD INTAKE

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Introduction

Over the last decade, the study of the impact of insect meal-based diets on growth performance and voluntary feed intake of several fish species have emerged, but little is known about their influence on fish appetite. Food intake could be regulated immediately after the first meal with a dietary treatment (short-term regulation), after several days of feeding (mid-term regulation), or after longer feeding periods (weeks, months, or years) reflecting the energy balance of the animal (long-term regulation) (Soengas, 2014). Understanding the underlying mechanisms responsible for central regulation of feeding behavior in fish, namely stimulation (orexigenic) or inhibition (anorexigenic) of appetite is crucial for proper formulation of aquafeeds and consequently ensure the best growth performance of fish. There are evidences that in fish, as in mammals, the key neuropeptides involved in orexigenic action are neuropeptide Y (NPY) and agouti-related protein (AgRP), while those involved in anorexigenic action are cocaine-amphetamine related transcript (CART), and pro-opiomelanocortin (POMC) (Soengas et al., 2018). According to our previous study (Basto et al., 2021), up to 80% fishmeal (FM) replacement by defatted *Tenebrio molitor* larvae meal (*d*TM) (36% *d*TM inclusion) does not affect central homeostatic regulation of food intake of European sea bass (*Dicentrarchus labrax*). However, the study was carried out after 10 weeks of feeding and possible changes in mechanisms involved in the short-and mid-term regulation of food intake remained unknown. In this context, this is the first study evaluating short- and mid-term homeostatic regulation of food intake in European sea bass fed diets not only with partial, but also total FM replacement by *d*TM.

Material and methods

A FM-based diet with 40% FM (47% crude protein) was used as control (CTRL) and two other diets were formulated to include 20% and 40% of *d*TM, replacing 50% (TM50) and 100% (TM100) of FM, respectively. After a 1-month acclimation period, fish were fasted for 24 hours before the experimental trial began to ensure that the basal levels of metabolites involved in the regulation of food intake were achieved. To evaluate the short-term response fish were fed for the first time with the experimental diets and sampled 2 and 24 hours post-feeding. To evaluate the mid-term response fish were fed three times a day and were sampled 2 and 24 hours post-feeding on the seventh day of experiment. In both short- and mid-term trials food intake was registered; hepatic and plasmatic metabolites and the expression of hypothalamic and telencephalic neuropeptides involved in food intake (NPY, AgRP, CART and POMC) were assessed.

Results

The dietary inclusion of *d*TM did not significantly affect fish food intake, neither at short- nor at mid-term. Accordingly, at central level, dietary treatments did not alter the expression of neuropeptide Y (*npy*), agouti-related protein 2 (*agrp2*), pro-opio melanocortin a (*pomca*) or cocaine- and amphetamine-related transcript 2 (*cartpt2*) in hypothalamus and telencephalon, neither in the short- nor in the mid-term. Despite no changes were observed in neuropeptides, the dietary inclusion of *d*TM induced some alterations in hepatic and plasmatic metabolites levels, either in the short- or mid-term. In the short-term, fish fed diets with total FM replacement by *d*TM had the highest glucose levels in the liver, whereas the α-amino acids were the lowest. After 7 days of feeding, specifically 24 hours after feeding the last meal, fish fed TM50 had significantly higher levels of glucose in liver than those fed CTRL and TM100, whereas hepatic triglycerides levels were significantly increased when fish fed TM100, irrespective post-feeding sampling time. Still in the mid-term response, the levels of α-amino acids in liver did not change with dietary treatment nor post-feeding sampling time. But 2 hours after feeding, the α-amino acids levels in plasma of fish fed TM100 were significantly lower than those fed CTRL and TM50.

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Discussion and conclusion

The results of the present study showed that the partial and total replacement of FM by *d*TM did not change food intake of European sea bass. The mRNA abundance of neuropeptides involved in the homeostatic regulation of food intake in both the hypothalamus and telencephalon remained unaffected in the short- or mid-term. Though, several changes in hepatic and plasmatic metabolites levels were observed, which may have negative effects on growth performance and/or health status of fish at the long-term. Thus, further studies are necessary to clarify such possible impact on intermediary metabolism of fish to provide further support for the use of *d*TM as a relevant ingredient in the formulation of more sustainable aquafeeds for European sea bass aquaculture.

Acknowledgements

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OPTIMIZATION OF ROTIFERS FEEDING PROTOCOL WITH *Nannochloropsis* sp. (PHYTOBLOOM® GREEN FORMULA)

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Introduction

Rotifers are essential for the nutrition of marine fish during their early life stages. However, rotifers nutritional requirements are still poorly understood. It is known that *Nannochloropsis* sp. is a microalgae species that promotes high growth efficiency of *Brachionus plicatilis* when adequate environmental conditions are provided. The expansion of the aquaculture sector has led to the intensification of rotifers production, thus increasing microalgae requirements in nurseries. Consequently, the use of industrially produced *Nannochloropsis* sp. products for rotifers nutrition expanded in the past decade (e.g. Phytobloom® Green Formula). The efficacy of the products on rotifers growth depends not only on the microalgae product biochemical profile, but also on its daily dosage adjusted to the rotifers concentration, mode of production and type of aquaculture system. The increase of the daily dosages must be conducted while monitoring systematically the quality of the water in order to establish mitigation strategies and to avoid potential deterioration of the water quality, which is responsible for the reduction of growth efficiency of the population. The objective of this study was to optimize the daily dosage of microalgae liquid concentrate of *Nannochloropsis* sp. (Phytobloom® Green Formula) in rotifers culture in a batch system.

Material and Methods

Different doses of commercial *Nannochloropsis* sp. liquid concentrate (18% dry weight) (Phytobloom® Green formula, Necton, Portugal) were applied in rotifers culture under batch production mode (4 days). Rotifers were cultured in 1.2m^3 cylindroconical tanks with aeration and oxygen with a temperature of 28ºC, 20ppt of salinity and a pH between 7.5-8. Rotifers concentration and percentage of rotifers with eggs were evaluated daily and the dosage adjusted according to the total number of rotifers in the tank. The environmental quality parameters were evaluated daily and the number of purges were increase from 2 to 3 during the experiment and applied throughout the day to remove the debris and maintain high water quality. The different feeding dosages were applied in several batches of rotifers (3-8) to evaluate the efficiency of each dietary protocol (Table 1).

Results and Discussion

The feeding protocols 4, 6 and 7 improved significantly the total number of rotifers obtained in comparison to protocols 1 and 2 (Figure 1A). There were no significant differences in the percentage of rotifers with eggs between treatments (Figure 1B). The 3rd day of batch presents significantly higher number of rotifers when compared the 1st and 2nd day of batch. Rotifers growth was significantly higher in the 4th day of batch when compared to the other days.

Throughout the experiment it was possible to observe that with the increase of the feed dosage there was a slight increase on the ammonia levels. This effect was strategically mitigated by the increase of the number of daily purges (2 to 3). Therefore, the optimization of the rotifers feeding dosages should be performed with a high control of the environmental parameters such as ammonia levels, and with the application of methodological strategies. Moreover, the control of the ammonia levels in rotifers culture is essential to avoid the inhibition of reproduction (3mg/L) (Schlüter & Groeneweg, 1985) and potentially culture crash. The application of the protocols 4, 6 and 7 improve the total number of rotifers obtained in comparison with the previously established protocol (dosage 1). In conclusion, the present study allowed to improve the application of industrially produced microalgae diets for rotifers in high density cultures for aquaculture.

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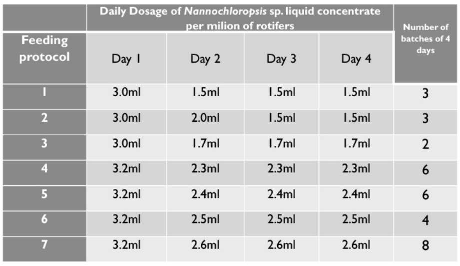
Table 1- Dietary protocols applied in rotifers culture under batch production mode with *Nannochloropsis* sp. liquid concentrate (Phytobloom® Green formula, Portugal).



Feeding protocol	Daily Dosage of *Nannochloropsis* sp. liquid concentrate per milion of rotifers				Number of batches of 4 days
	Day 1	Day 2	Day 3	Day 4	
1	3.0ml	1.5ml	1.5ml	1.5ml	3
2	3.0ml	2.0ml	1.5ml	1.5ml	3
3	3.0ml	1.7ml	1.7ml	1.7ml	2
4	3.2ml	2.3ml	2.3ml	2.3ml	6
5	3.2ml	2.4ml	2.4ml	2.4ml	6
6	3.2ml	2.5ml	2.5ml	2.5ml	4
7	3.2ml	2.6ml	2.6ml	2.6ml	8

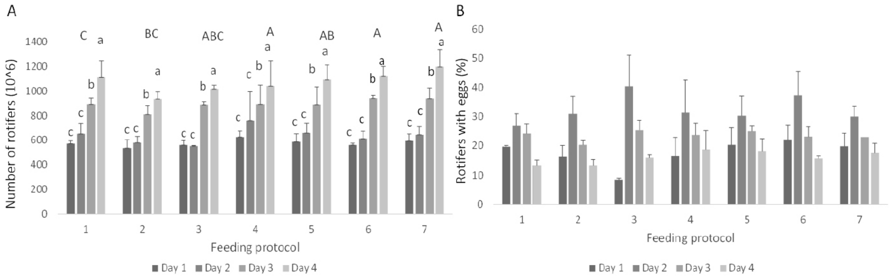


Fig 1– Rotifers growth efficiency with different feeding protocols (dosages) with *Nannochloropsis* sp. liquid concentrate produced under batch production mode regarding A) total number of rotifers and B) percentage of rotifers with eggs. Data is expressed as means and standard deviation. Significant differences between the number of rotifers throughout time in each treatment are expressed by low case letters while significant differences between dietary protocols are represented by different upper-case letters (two-way ANOVA, post hoc Tuckey $p<0.05$).

Acknowledgements

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Bacillus indicus AND *Bacillus subtilis* AS AN ALTERNATIVE HEALTH SUPPLEMENT TO ASTAXANTHIN

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Introduction

Infectious diseases are one of the biggest burdens in the growing aquaculture industry (Assefa and Abunna, 2018). Globally, antibiotics are widely used to treat and prevent bacterial diseases. However, due to increasing prevalence of antibiotic resistance, their use can potentially severely harm the environment, humans, and/or reduce treatment efficiency (Dawood, Koshio and Esteban, 2018)). Therefore, novel alternative strategies to improve fish health and increase disease resistance are urgently needed. The carotenoid astaxanthin is a widely used feed supplement with well-known health benefits for the host and used as a/or colourant for fish flesh/skin. However, due to high productions cost, its use is restricted to high-value species (Lim *et al*., 2018). In the following experimental study, we are testing a novel two-species probiotic - *Bacillus indicus* and *B. subtilis* - as a potential alternative health supplement to astaxanthin in mirror carp (*Cyprinus carpio*). The main goal of the study was to use experimental feed trials and 16 S rRNA microbiome profiling to compare the effects of B. indicus and traditional astaxanthin supplementation on fish performance and health, based on the overall microbial community of the gastrointestinal tract.

Materials and methods

Mirror carp (*Cyprinus carpio)* with an average weight of 40 g were fed for 7 weeks in triplicate groups on one of three experimental diets (negative control: no supplement, probiotic: 0.36 g/kg *B.indicus* + 1 g/kg *B.subtilis,* positive control: 40mg/kg astaxanthin). Fish were raised in a recirculation aquaculture system (RAS, 12 x 200L tanks, Pontus Research) at 21 °C and 12:12 h light: dark regime. Batch weights for fish growth performance were taken throughout the trial (weeks 0, 4, and 7). At the end of the trial blood samples were taken from 5 fish per tank (20 per treatment) for haematological and immunological analysis. For the microbial analysis, a swab sample from the distal intestine of the carp was taken from 3 fish per tank (16 per treatment group). The swab samples were processed at Bangor University following standard procedures of 16S rRNA metabarcoding. Sequencing data were processed in the quime2 pipeline and results were tested for significance using R software packages.

Results

Over the 7-week carp trial, fish fed the probiotic showed a greater specific growth rate (SGR) compared to the control, however not significant. Fish fed astaxanthin demonstrated significantly increased SGR. The haematological and immunological analysis showed significant results in fish fed the probiotic (e.g., increased phagocytic activity). In carp, we found that both treatment groups significantly increased microbial diversity in the distal intestine (Figure 1). The probiotic increased the abundance of potentially beneficial bacteria including *Bacillus spp*.. Overall, the probiotic blend induced a wider community shift compared to astaxanthin. Out of all (37) significant differentially abundant genera, 51.4 % (19) are shared between both supplements, while 45.7 % (17) of the genera are only differentially abundant in the probiotic. One genus was exclusively found more abundant in Astaxanthin. In addition, a greater number of bacteria metabolic pathways were altered in carp fed the probiotic compared to astaxanthin. However, during the microbial analysis, the probiotic bacteria species could not be detected in the distal intestine.

Conclusion

The probiotic supplement *B.Indicus*/*B.subtilis* significantly increased gut microbial diversity in carp, similar to astaxanthin supplementation. However, the probiotic induced greater changes in abundance across the microbial community. Increased microbiome diversity is likely linked to healthier fish (Li *et al*., 2017; de Bruijn *et al*., 2018) for example, our bloodwork analyses demonstrated significantly increased phagocytic activity in fish fed the probiotic, indicating an increased immune response. Despite changes in the microbial community, we could not specifically detect the probiotic bacteria species in the gut region studied. This suggests that the probiotic could not colonise the distal intestine of this host species. However, based on the significant outcomes in fish fed the probiotic, it is possible the probiotic is established in a different part of the gastrointestinal tract, or alters the host gut microbiome during its passage through the gut. Overall, we conclude that the *B.inidicus/subtilis* probiotic has great potential to be used as a replacement for astaxanthin. However, further research is required to understand the mechanisms of *Bacillus spp*. probiotic establishment in fish gastrointestinal microbiomes, and the consequences for fish health.

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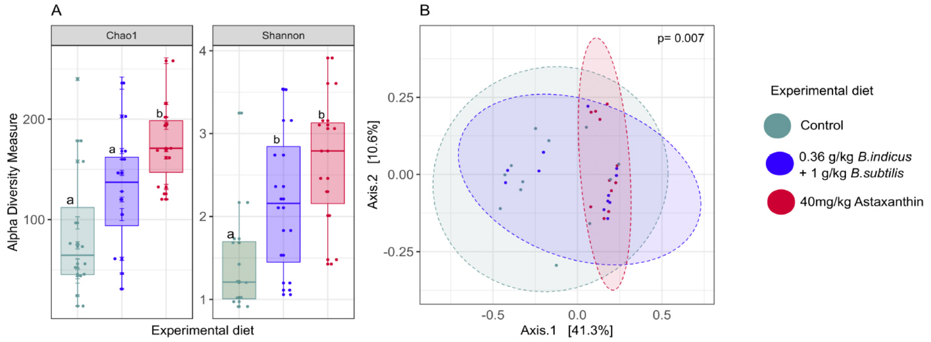

Figure 1: Diversity measures of the microbial community in carp (A & B) under probiotic inclusion levels and the supplementation of astaxanthin (green = no supplement [control], orange = 0.5 g/kg, purple = 1.0 g/kg, 2.0 g/kg), Alpha diversity measured by Chao1 and Shannon indices. NMDS ordination of Beta diversity communities. Ellipses indicate 95% confidence.

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SOCIO-ECONOMIC CHALLENGES OF THE AQUACULTURE SECTOR IN THE ATLANTIC AREA AND THE POTENTIAL OF INTEGRATED MULTI-TROPHIC AQUACULTURE AS A SUSTAINABLE APPROACH: ASTRAL PROJECT

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Introduction

Consumption of seafood has grown twice as fast as the population since the 1960s (OECD[1]). When facing with this rising demand, aquaculture stands-out as a response to the need to increase aquatic and seafood production. In fact, according to FAO, world aquaculture production reached an all-time high of 114.5 million tons with a total farm gate sale value of USD 263,6 billion in 2018. This aquaculture growth all over the world and its intensive production causes a significant set of problems (i.e., creation of an anoxic zone, reduction in water quality, destruction of habitat, use of antibiotics, amongst others). In order to meet these demands, the sector must develop innovative, responsible and profitable cultivation methods to optimize efficiency and reach societal requirements. In this context, the Integrated Multi-Trophic Aquaculture (IMTA) farming is a promising system to tackle some of the aquaculture challenges to build up a more sustainable blue economy; in that regard, the EC has funded some projects and the Horizon 2020 project, ASTRAL, is one of them. ASTRAL focuses in IMTA farming as a possible alternative to develop a sustainable, profitable and resilient aquaculture. The project objectives include defining, supporting and promoting IMTA production across the Atlantic area and to identify new value chains. A socio-economic assessment is key to support those objectives and highlight challenges and barriers of aquaculture and the IMTA possible solution approach; here are some of the preliminary results and perspectives of the project.

Methodology

The socio-economic analysis aimed to draw up a state of the art of the aquaculture in the Atlantic area, especially in the ten ASTRAL involved countries: Norway, Spain, France, Portugal, Ireland, United Kingdom (Scotland), Brazil, Argentina, South Africa and Nigeria. This study highlights the main characteristics of the aquaculture sector and the main challenges that it faces. It gives key elements to understand the aquaculture context and provides a framework to enable as a funnel to refine the analysis level.

The methodology used was based on:

- the territory and regional knowledge (macro and meso economic approach) thanks to a strong bibliographic review per country,
- producers' knowledge (meso and micro economic approach) thanks to a survey and preliminary results of producers' one-to-one interviews.

Technology and Innovation

Technology developments and advancements have modernized and upgraded the processes efficiency, product quality and competitiveness in the aquaculture industry. However, access to the latest technology and practices for aquaculture farmers in some countries remains a challenge due to a lack of information, training and capital. The ASTRAL project assesses 6 technologies that are deployed in IMTA systems to identify the systems needs and added value of the species combination throughout the production cycles, developing operation welfare indices (health management), assessing risk of pathogen infection (biosecurity) and profitability. The knowledge transfer will be beneficial to suggest new and suitable IMTA combinations considering the regional challenges.

Economic challenges

In an increasingly competitive environment, farmers must enhance efficiency and profitability when using economic resources. Owning an aquaculture facility can be expensive because of the structure costs to fully develop aquaculture businesses differ depending on aquaculture types and species as well as fixed and variable costs.

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Some IMTA research studies have shown economic advantages in comparison with non-IMTA systems, including increased physical production from a given site, greater diversity of production, and more intensive use of facilities, labour and sites (D. Knowler al., 2020). A more concrete study discounted cash flow analysis using 10 years of production data from a salmon-mussel-kelp IMTA farm in the Bay of Fundy, Canada. It found that the IMTA operation was more profitable than standard monoculture expectations and, if a 10% price premium could be obtained for the product, IMTA would produce a substantial increase in net present value (Eumofa, 2020). The advantages must be balanced with the potential economic disadvantages such as greater complexity in terms of marketing (outlets for the different species, operations, juveniles, business planning; and regulatory (potential increase of requirements), more risks, and greater challenges in the site selection. The ASTRAL project contributes to obtain data of this process and bring the experience of the five labs in Scotland, Ireland, Brazil, Southern Africa and Argentina.

Social challenges

Aquaculture plays a key role in urban and rural communities, and in local economies. The development of human resources both in quality and quantity is crucial to sustain the aquaculture industry in the future, especially in the context of increasing complexity ecosystems and changing paradigms. From the perspective of developing IMTA production, it is difficult to gather all the know-how required to cultivate several species within one system. The difficulty of mastering the cultivation of several species at the same time appears to be a major obstacle to the IMTA development. However, IMTA can be a good solution with regards to the social acceptability of aquaculture, once the concept is clearly explained. More social science research on aquaculture is needed, particularly to better understand the influence on consumers' perceptions.

Conclusion

The aquaculture sector is facing a lot of challenges that will need to be tackled in a near future: climate change, rising production costs, sustainable fish feed, working conditions and spatial planning. IMTA can be one of the options to limit these challenges by operating effluent biomitigation, optimizing energy in a production loop, promoting responsible businesses and optimizing the space availability/needs, to appear as a flexible approach according to the types of species, the production sites and the production systems.

However, IMTA is still facing barriers and meeting challenges that might explain its low development in the Atlantic area, such as resistance to change, complexity of setting-up, added costs and efforts and the lack of economic performance demonstration. Within ASTRAL we hope to contribute to this economic and societal assessment.

The ASTRAL project has received funding from the EU Horizon 2020 Research & Innovation Programme under Grant Agreement n°863034

Organisation for Economic Co-operation and development

PRESERVATION OF THE DONETS RUFFE *Gymnocephalus acerinus* POPULATION IN UKRAINE

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At present, 69 species of fish are included in the Red List of Ukraine, the reasons for this position are the disappearance of typical habitats and as a result of changes in hydrological, chemical, biological regimes of water bodies caused by hydraulic engineering construction; water pollution, accidental uncontrolled fishing by amateur fishermen.

In recent years, the problems of conservation of aboriginal species are paid great attention. In particular, this applies to Donets ruffe *Gymnocephalus acerinus* rheophilic species, preserved in some places in rivers where there are no hydraulic structures, for example in the Desna river basin. One of the effective ways to preserve this species is artificial reproduction in aquaculture and further restocking in natural reservoirs. In EU countries, such work is often done by National Parks, universities, research institutions and private enterprises, which work not only with commercial species, but also with small and endangered fish and developing methods for restoring aboriginal species populations in natural bodies of water. In practice, Ukraine has already had successful attempts to reproduce endangered species in artificial conditions, one of which is a set of research projects on the conservation of umbra (*Umbra krameri*) in Moldova and Ukraine, conducted by the Ecological Union “BIOTICA” with the support of the project “Conservation of Endemic European umbra in the lower parts of the Dniester-river”, supported by the British organization Flora & Fauna International. The experiments have been conducted since the early 2000s. According to this technology, fish were caught from natural reservoirs, placed in pre-prepared aquariums, which maintained the necessary hydrological regime for this species and created places for natural spawning, and then the resulting fry were released into the reservoir to create a local natural population.

Within the framework of the scientific program on artificial reproduction of the *Gymnocephalus acerinus* it is planned:
2.1 Low-traumatic catch of broodstock and juveniles from their natural habitats.
2.2 Timely delivery of harvested broodstock and fish of different ages to the center of aquatic bioresources and aquaculture NULES of Ukraine. Creation of artificial habitats close to natural ones.
2.3 Domestication (feeding live and artificial feed, preparation for spawning and artificial reproduction).
2.4 Carrying out artificial reproduction.
2.5 Growing fry and fingerlings to viable stages.
2.6 Stocking and restoration of natural populations in the native area.

At present time scientists of the National University of Life and Environmental Sciences of Ukraine have searched for the locations of *Gymnocephalus acerinus* in the Desna River and planned to catch and further domesticate it.

NON-INVASIVE (PHOTO) INDIVIDUAL FISH IDENTIFICATION OPEN ACCESS LARGE DATA BASE

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Introduction

The idea of the precision fish farming concept (Føre et al., 2018) is the automatization of the farming process, which can help control, monitor, and document the biological processes of fish cultivation. One of the optimization ways of the precision fish farming concept is finding an opportunity to get the individualized information about fish. The farmers could have personalized information about each fish in the tank (cage). Identification of individual fish could open a lot of possible solutions for the fish farmers. With the individualized information about fish, the processes of fish production could be more profitable. It helps deceases early detection, which could predict the high mortality. The identification of individuals could be a substitute for the fish tagging method (Andrews, 2004). This method is harmful and stressful for the fish. Fish has to be caught for identification which is time-consuming (Rácz et al., 2021). Non-invasive fish identification (photo-identification) is cheap and faster. The critical part of for individual fish identification method development is the large fish individuals dataset. We collected a large dataset of four fish species and provided it as an open access database for the researchers all over the world to play with and test their own methods on our data sets.. Those data sets were used for developmentof our own approaches for the identification of fish individuals. With the use of those data, three papers were published. One paper (European seabass and common carp) is under submitted status.

Materials and methods

Database is available on our data management platform – bioWES (http://www.biowes.org/). bioWES is a platform for experimental data and metadata management. General protocol (individual fish identification) obtains four different protocols for four fish species. Dataset of four fish species were collected,: Atlantic salmon *Salmo salar*, Sumatra barb *Puntigrus tetrazona*, European seabass, and common carp (Fig.1). Each protocol has two subprotocols. One is a protocol with the original data (data collection) and another one is protocol with the processed data. Some protocols end with the final protocol which have a final paper which was published according to the used data. Below are chapters with the description of four experiments (protocols).

Atlantic salmon - A total of 328 fish were used for short-term identification to test the identification power of the pattern. Thirty of the fish were tagged with PIT tags and used for long-term identification to test pattern stability. A total of 4 data collections were performed over six months at two months intervals. Three types of data were taken in each session: lateral view images of the fish out of the water (in a photographic tent) and underwater (in a small aquarium) and iris of the fisheye. The NIKON D90 digital camera was used to take approximately eight images of each fish in RAW format. Images of the 30-tagged fish were also collected as the long-term dataset during the next six months. The regions of interest (ROIs) were automatically extracted for all images in all datasets using image processing methods.

Sumatra barb - 25 fish individuals of Sumatra barb were used in this experiment. The digital camera with controlled lighting, the background, and the fish position, was used for data collection. Data were collected under different angles and positions; images were taken from one side view of all fish. Data were collected two times during two weeks for fish inside the aquarium. Tree images of each individual were taken in every data collection. The fish detection procedure consists of standard image segmentation based on color subtraction, object detection, filtration, and parametrization.

European seabass - Totally 300 sea bass were used during the experiment. All 300 fish were used for the testing the uniqueness of the visible patterns of the fish for identification of the individuals (short term experiment (ST)). Randomly selected 32 fish individuals out of the 300 fish were tagged by PIT tags for the long-term experiment (LT) to test the stability of chosen patterns during the cultivation period. After two months, the second data collection was performed. Four different ROI (regions of interest) were used for identification. All ROIs were detected automatically in the fish image.

Common carp - Four rounds of data collection were performed over the four-month experiment for carp identification. Thirty-two fish individuals were used in this experiment. Images were taken of the whole fish out of water. Each fish was caught in the cultivation tank, anesthetized in a bucket using clove oil, and moved to the green background for imaging. Automatic data processing consisted of three steps: fish detection, region of interest (ROI) selection, and feature extraction to describe the skin patterns of the ROI.

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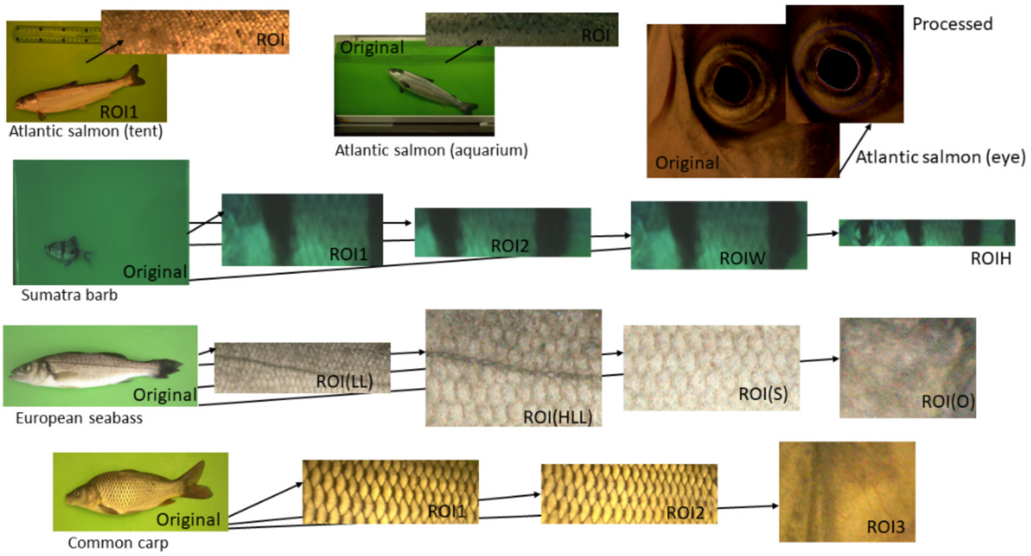

Figure 1. The data base examples. Original data and processed data of four fish species.

Results and discussion

The results of those experiments are fully presented in our papers. The datasets are scaled and focused on the different aspects of fish identification. The first dataset contains images of Sumatra barb. The methods of identification can be first tested on this dataset because of obvious stripe pattern on the fish and data close to the real conditions. We reached the the classification accuracy of 100%. More information is presented in the paper of Bekkozhayeva et al. (Bekkozhayeva, Saberioon, & Cisar, 2021). As a second datasetis the salmon data. Salmon also was strong dot patterns on the body but this dataset contaoins significantly more individuals. This identification resulst results had been described in the paper of Cisar et al. (Cisar, Bekkozhayeva, Movchan, Saberioon, & Schraml, 2021) and Schraml et al. (Schraml et al., 2020). Once the methods are successful dot strongly visinle pattern it can be tested on dataset for carp and sea bass. These fish species has only the scale pattern or lateral line patter which can be used for identification. But the results of European seabass and common carp are not published yet. The paper with the experiments and the results is under submission. In general, those studies demonstrated that the image-based individual fish identification is accurate and could be used for individual fish identification as a substitute for the tagging methods. The pattern stability was proved together with their stability.

Acknowledgments

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NON-INVASIVE GENETIC TAGGING OF EUROPEAN LOBSTER JUVENILES FOR STOCK ENHANCEMENT PROGRAMS

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Introduction

Parentage based tagging (PBT) is a valid approach for monitoring the effects of conservation-oriented stock enhancement programs. However, collecting samples for DNA extraction from live decapod juveniles could be challenging, especially during the first developmental stages. In this context, PTB is usually carried out by analysing egg subsets, to reconstruct genetic profiles of siblings. However, this strategy prevents individual-based analyses and monitoring programs (e.g. analyses of survival and dispersal patterns). In this regard, a non-invasive method is required for individually tagging hatchery-reared juveniles designed for stocking programs. Here, we propose a novel, non-letal and non-invasive approach to PBT of *H. gammarus* juveniles, which allows individual genetic tagging from exuviae, overcoming the typical constraints of traditional methods.

Materials and Methods

We analysed individual exuviae obtained from hatchery-reared IV-V stages of *H. gammarus*. 10-20 ng of tissue suitable for DNA extraction were collected from the branchial chamber left inside the exuvia. Sampling was performed randomly, without knowing individual kinship. Three DNA extraction methods were employed: (i) CTAB, (ii) Quick DNA miniprep kit of Zymoresearch, (iii) HotSHot (Montero-Pau *et al*., 2008). Quantity and quality of genomic DNA were assessed by fluorimetric analysis. Extracted DNA was amplified at 12 microsatellite loci (Andrè and Knutsen 2010, Ellis *et al*., 2015). Quality checks of microsatellite data and parentage analyses were carried out using CERVUS (Kalinowski et al., 2007) and COLONY (Jones & Wang, 2010) softwares, respectively.

Results

Genomic DNA suitable for genotyping and parentage analysis was successfully extracted from all three methods. 10-50 ng/μL, 8-22 ng/μL and 14-26 ng/μL of DNA were obtained by using CTAB, commercial kit and HotSHOT method respectively. The 260/280nm ratio was 1.6-1.7, 1.8-1.95 and 1.96-2.09 for CTAB, commercial kit and HotSHOT method respectively. Microsatellite amplification and sequencing was successful for the entire set of 12 SSRs, and parentage analyses allowed to reconstruct mother-offspring relationships with high confidence for all the individual lobsters analysed.

Discussions

This study demonstrates that it is possible to obtain good quality genomic DNA from the exuviae of juvenile lobsters, in a sufficient amount for parentage analysis based on a set of 12 SSR. By allowing the identification of individual lobsters after their release in the wild, this novel approach will open the door to long-term monitoring programs of survival and dispersal patterns. Moreover, considering the good performance of all DNA-extraction methods, we anticipate that this approach could be also useful in post-release monitoring programs of a wide range of decapod crustaceans.

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DOWNSCALING COPERNICUS ERA5 WAVE DATA TO IDENTIFY SUITABLE MARINE AREAS FOR MEDITERRANEAN AQUACULTURE

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Introduction

Lack of marine space and suitable sites for fish and shellfish farming is recognized to be one of the most relevant factor burdening the expansion of mariculture (COM 2021/236 final). The suitability of marine areas for the installation of aquaculture facilities depends on several factors. Among these, the wave climate plays a relevant role, both in terms of average conditions and extreme events. Unfortunately, direct wave measurements are only available at very few points along the coast and it has become widely accepted that a suitable methodology is to employ databases of hindcasted wave conditions, validated and calibrated against available *in situ* measurements. However, these hindcast datasets typically have a low resolution, not adequate to be used in spatial multi-criteria evaluations finalized to identify the most suitable areas for aquaculture (Marino et al., 2020). In order to produce a high-resolution dataset, the results of the global scale hindcast models is used to drive regional and local computations. The procedure, inspired to the modelling chain described in Bellotti et al. (2021), is also known as "downscaling". In this paper, we present an example application of such procedure, starting from the global dataset ERA5, on the south-central Tyrrhenian Sea nearshore areas (Fig. 1) and its use for a Weighted Linear Combination (WLC) analysis.

Materials and methods

The global dataset used to feed the nested model grids is the ERA5 (Hersbach et al., 2020), produced by the ECMWF (European Centre for Medium-Range Weather Forecasts) and freely available in Copernicus (hourly hindcasts since 1950). The selected wave model is the open-source SWAN (Simulating WAves in the Nearshore), developed by Delft Technical University. One single computational grid (Fig. 1) was used to model the wave propagation from the offshore boundary, mostly aligned with the available ERA5 grid points position, up to the coast. Validation and calibration of the model was carried out with reference to the direct wave measurements available in the area (4 wave buoys and satellite products). From the wave time series with information on spectral significant wave height (Hm0) of the computational grid, an "inverse distance weighting" (IDW) interpolation was used to have a regular grid with a resolution of 750m for each cell and within 12 nautical miles from coastline. The percentage of events in which the value of Hm0 fell within optimal suitability ranges for fish (<0.7m) and mussels (<0.2m) was calculated (Marino et al., 2020). A suitability index was assigned considering the observations in the optimal range.

Results

A very good agreement resulted between wave data observations and modelling results as showed for the selected area of Gulf of Naples (Fig. 2), suggesting that the results can be safely used for the present purposes. The wave time series produced by the downscaling procedure and the classification according to defined optimal suitability criteria for fish and mussels, returned two GIS layers (raster format) representing the suitability of marine areas for aquaculture against wave climate (Fig. 3).

Discussion

Robust high-resolution datasets are essential for a correct aquaculture zoning process and to identify marine sites suitable for wave climate and at low risk of extreme events. SWAN model reproduced reliably most of the phenomena of importance in the nearshore of study area. Wave analysis, together with other oceanographic, biogeochemical and marine uses criteria, allows the identification of suitable marine sites for fish and shellfish farming.

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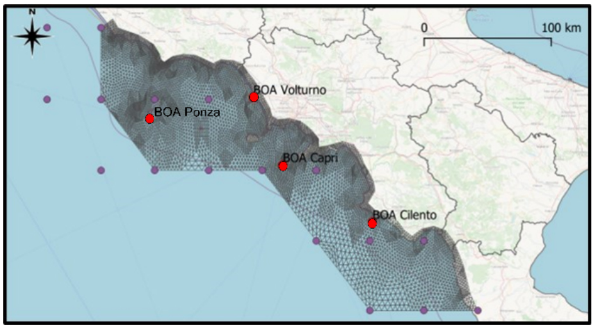

Fig. 1 Finite element computational grid for the SWAN wave model. Purple dots indicate the position of the available ERA5 wave data and red dots indicate wave buoys.

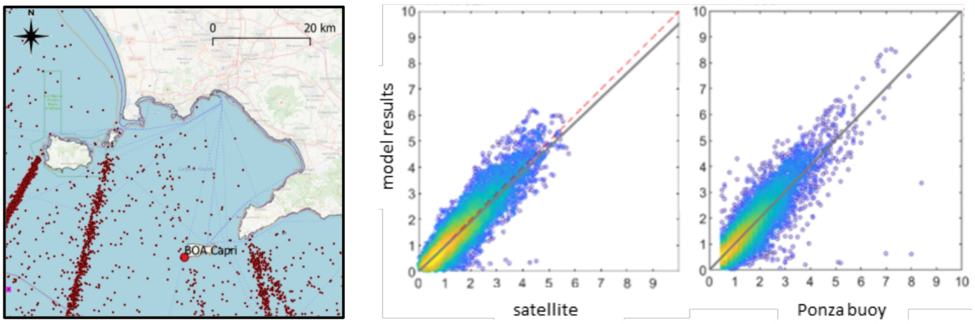

Fig. 2 Validation of the wave model results. Left: position of the available satellite wave measurements in proximity of the Napoli Gulf. Middle: comparison between satellite and wave model significant wave height. Right: comparison between Ponza buoy and wave model significant wave height.

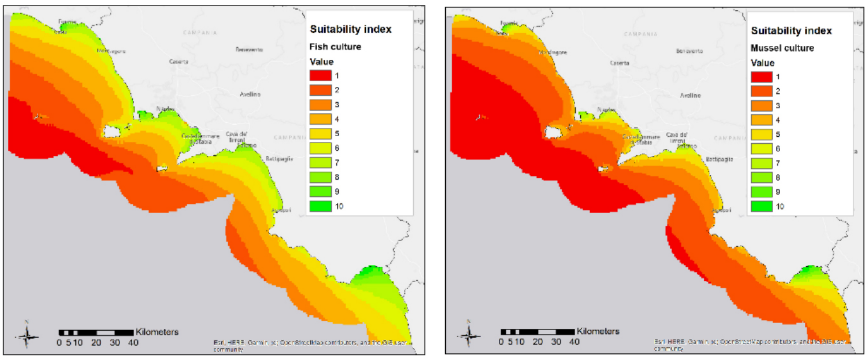

Fig. 3 Suitability maps of wave climate for fish (left) and mussels (right) aquaculture in Campania.

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OVERVIEW ON ADVANCED TECHNOLOGIES OF NEW MARINE FISH SPECIES AND CHALLENGES LIMITING THEIR COMMERCIAL VIABILITY FROM HATCHERY TO OFFSHORE AQUACULTURE

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Introduction

Aquaculture technology for several commercially important species of tropical and sub-tropical marine fish from hatchery to growout to market has become or is quickly becoming available. Hatcheries are capable of producing juveniles of candidate species for offshore aquaculture such as cobia (*Rachycentron canadum*), hamachi/kampachi (*Seriola rivoliana, S. lalandi*/*S. dorsalis*), pompanos (*Trachinotus carolinus*), snappers (*Lutjanus guttatus, L. peru and L. campechanus*), yellowtail snapper (*Ocyurus chrysurus*), totoaba (*Totoaba macdonaldi*), red drum (*Sciaenops ocellatus*), mahi (*Coryphaena hippurus*), tripletail (*Lobotes surinamensis*) – among others. Steady supply of high-quality juveniles of certain species is still limited, but it is unlikely that this will remain a bottleneck for industry expansion. Likewise, offshore technology is developing fast and advanced systems are already available.

Overview and Challenges

Land-based recirculating aquaculture systems (RAS) and traditional flow-through ponds, raceways and tanks are all viable options. However, large scale production required to feed the world in the next decades will have to be produced in the open ocean - where stronger currents and greater depths increase the carrying capacity of the environment. Raising fish in exposed, high-energy areas offshore require advanced technologies demanding high levels of investment and long-term commitment. Hence, fish produced offshore must be sold at high prices to compensate the high capital and operating costs required, limiting their demand in a highly competitive white fish market.

Offshore aquaculture continues to expand the world over, yet the commercial viability of operations remains mostly elusive. Infrastructure and logistics are in place, and market demand is rising. Technology continues to expand rapidly. Tools for site assessment and selection and environmental monitoring have been established. Hurdles still must be overcome before commercial viability can be secured. Some issues such as optimizing genetics, nutrition, and diseases control are inherent to all forms of aquaculture - whereas managing stocking, feeding, chemical treatments, net cleaning, predator avoidance, escapements, biomass estimates and crop management, mortalities collection and harvesting operations are exacerbated in offshore systems. Full automation is progressing fast but still needs refinement. Machine learning and artificial intelligence tools are becoming available and being incorporated to perfect systems automation. The development of practical, specialized feeds for all developmental stages of selected species remains a challenge. These issues are presented and addressed in this presentation.

Results and Discussion

We present and discuss these challenges and how the industry is collectively working with researchers to address and resolve issues limiting the technological and commercial viability of new species with regards to hatchery and offshore aquaculture.

FIRST-FEEDING DIETS FOR EUROPEAN EEL LARVAL CULTURE: INSIGHTS AT MORPHOLOGICAL, NUTRITIONAL AND MOLECULAR LEVELS

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Introduction

Closing the life cycle in captivity of European eel is a requirement to develop sustainable aquaculture of this species. Due to the decline of the population together with low levels of juvenile recruitment, this species has become critically endangered and subject to trade restrictions (Pike et al., 2020). Thus, hatchery production of offspring is essential in order to lift limitations on aquaculture production and markets for European eel (Tomkiewicz, 2019). Information regarding the eel early life stages is scarce, which has considerably delayed the development of culture techniques and technologies. The main bottleneck for progressing in rearing hatchery-produced eel larvae is the establishment of an adequate first-feeding regime. First studies showed that European eel larvae successfully ingested a paste made of rotifers, *Brachionus plicatilis* (Butts et al., 2016), but despite successful initiation of first-feeding at 15 dph (cultured at 18°C) and an increased body area, indicating growth compared to starving conspecifics, the larvae did not survive past 24 dph (Politis et al., 2018). However, the latter study showed thatInterestingly though genes encoding the major digestive enzymes were expressed earlier than the actual initiation of first feeding (already at 4 dph), indicating a premature molecular functionality initiation of the gastro intestinal tract. Subsequent studies have focused on the possibility to promote an early maturation of the gut by early introduction of feed, where it was indicated that early availability of dietary nutrients can influence the molecular ontogeny of feeding related mechanisms and processes, but the successful initiation of larval feeding depends on the quality and attractiveness of the diet(s) (Benini et al., 2022). In the present study, three diets were formulated and experimentally tested as potential first feeding prototypes for hatchery produced European eel larvae. Larval mortality was recorded daily from the onset of the first feeding stage at 10 dph until 28 dph, while sampling was conducted at regular intervals to obtain larval biometrics and gene expression patterns to follow the molecular ontogeny of digestive functions and growth of the larvae during the experimental period.

Material and methods

Broodstock management, gametes production and egg incubation was performed according to Kottmann et al. (2021) and culture of the yolk sac larvae were performed according to the protocol described in Benini et al., (2022) until they reach the first feeding stage. When reaching the first-feeding stage, eel larvae were divided into replicated Kreisel tanks (n = 9) of 8 L capacity each, at a density of ~60 larvae/L and randomly connected to three separate, but identical 0.65 m^3 RAS units, where the water was kept at 18 psu and ~20°C, while light (~500 lux) was only turned on during feeding (Benini et al., 2022, Politis et al., 2021, 2017). In each Kreisel tank, larvae received feed at a concentration of 0.5 mL diet per L of water five times per day at two hour intervals. Here, light was turned on and water current was stopped to give larvae the possibility to feed on the diet, which was directly pipetted on the bottom of the tank. Larvae were allowed to feed for 30 min, subsequently the light was turned off and water flow resumed. The remaining feed was flushed away using a gentle jet of water. Larvae were moved into clean tanks daily. The three diets were freshly prepared daily. Diet 1 contained spiny dogfish (*Squalus acanthias*) egg yolk, krill extract and soybean peptide, while Diets 2 and 3 represent modifications of Diet 1. Those two diets had a proximal composition of 60% protein and 30% lipid, but the protein source and molecular size differed. Diet 2 contained fish hydrolysates, which consisted of peptides of approximately 3 kDa in size while Diet 3 contained whey (by-product of cheese making), with a molecular protein weight of 10-12 kDa. Larval cumulative mortality was calculated as a percentage from 10 until 28 dph. Moreover, pools of larvae per replicated tank (n = 3) and diet (n = 3) were sampled at selected developmental stages (0, 9, 15, 22 and 28 dph) for biometrical measurements, dry weight calculations and gene expression analyses.

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Results and Discussion

Results showed that eel larvae successfully ingested all three diets with the overall feeding incidence increasing from 37.8% to 54.4%. Gut fullness increased from 20.03% to 24.9% between 15 and 22 dph, with the highest gut fullness registered for Diet 3. Independently from the diet, two periods of high mortality were identified. The first appeared shortly after introduction of feed (10-12 dph), while the second, occurred at 20-24 dph, indicating that the larvae reached the "point of no return". The latter interpretation was supported by continuous upregulation (peaking at 22 dph) of the gene ghrelin (*ghrl*), the so-called "hunger hormone" in all dietary trials indicating that most eel larvae were fasting. However, only in larvae fed Diet 3, the expression of *ghrl* was downregulated beyond 22 dph, indicating that those larvae were not starving. Concurrently, differential upregulation of genes encoding the major digestive enzymes (*try*, *tgl*, and *amyl2a*) at 22 dph for larvae fed Diet 3 supported their prosperity at the molecular level. Furthermore, the expression patterns of those, together with food intake (*pomca*), and growth (*gh*) related genes, continued to increase towards 28 dph for larvae fed Diet 3, while we have no data for larvae from Diet 1 and 2, since very few larvae survived beyond 22 dph. These results together with an improved survival, increased dry weight, and growth in length and body area underline that Diet 3 possesses essential nutritional components, resulting in an improved maturity and functionality of the molecular feeding, digestion, and growth mechanisms. Nevertheless, we observed an early benefit of Diet 1 regarding higher survival, enhanced morphology (length and body area) and gene expression (*amyl2a*, *tgl*, *pomca* and *gh*) but, overall, the inclusion of more complex dietary proteins as in Diet 3, but not hydrolysed peptides as in Diet 2, seems to be beneficial for a successful transition to exogenous feeding. Thus, we conclude that the digestive and assimilation capacity of European eel larvae varies throughout ontogeny and consequently, the dietary regime might need to be stage specifically adapted. Overall, the results of the present study provide an unprecedented step towards establishing a first feeding culture of hatchery produced European eel larvae.

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DO FISH ESCAPEES INFLUENCE WILD FISH MARKET PRICES? AN ANALYSIS IN THE SPANISH MEDITERRANEAN

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Introduction

Spain is the main producer and consumer of the seafood in EU, covering over 45% of the total seafood volume consumed and 23% of the total aquaculture production in 2019 (APROMAR, 2021). Indeed, aquaculture is a well-developed sector in the Spanish blue economy, but at the same time, escapees from fish farms are also a source of environmental pressures and economic externalities. The risk of fish escape incidents depends on farmed fish biology, sea storms intensity and farming equipment and operation failures (Jensen, 2006). These events have a significant effect on the local fisheries generating high landings in short periods (Toledo-Guedes et al., 2014). Despite escapees can influence fish abundance, their potential market effects have received little attention in the literature. In fact, there is not quantitative research aimed to estimate to what extent landing anomalies caused by escapees influence wild fish prices. The objective of this study is to analyze the economic effect generated by escaped fish in the local seafood market, in particular, in the first sale prices of seabass (*Dicentrachus labrax*), seabream (*Sparus aurata*) and meagre (*Argyrosomus regius*).

Material and methods

This research uses panel data analysis to quantify the effect of landings anomalies caused by escapees on wild fish prices. Time series of prices and landings for 24 Spanish Mediterranean harbor markets located in Comunidad Valenciana and Murcia for the period 2004-2021 are extracted from Ministry of Agriculture, Fisheries and Food. Anomalies in landings are identified from landing data using R Studio software, (package “anomalize”). Other control variables related to production costs (Castillo-Manzano et al., 2015), presence of fish farms and seasonal factors are included in the model (Toledo-Guedes et al., 2014). The empirical model is defined as follows:

$$\mathrm{Price}_{i,t} = \beta_0 + \beta_1\,\mathrm{LANDINGS}_{i,t} + \beta_2\,\mathrm{ANOMALY}_{i,t} + \beta_3\,\mathrm{OILPRICE}_{t} + \beta_4\,\mathrm{LABOURCOSTS}_{t} + \beta_5\,\mathrm{VESSELS}_{i,t} + \beta_6\,\mathrm{INSTALLATIONS}_{i,t} + \beta_7\,\mathrm{DISTANCE}_{i,t} + \beta_8\,\mathrm{S1} + \beta_9\,\mathrm{S2} + \beta_{10}\,\mathrm{S3} + u_{i,t}$$

Stationarity of time series was tested using the unit-root Dickey-Fuller test. Following Hausman tests, the fixed effect estimator was selected and estimated independently for each specie. Several regression diagnostics were run to obtain variance inflation factors, and to test for serial correlation and heteroskedasticity (Greene, 2018).

Results

Table 1 presents regression results, including only those variables that were statistically significant. All variables present the expected signs, showing that prices are inversely related to landings and anomalies. In average terms, anomalies decrease prices of seabass, seabream and meagre by 4.18 €, 1.9 € and 2.17 €, respectively. The presence of fish farms also seems to have effects on prices, given the direct relationship between distance and prices. It suggests that aquaculture may influence fish abundance and prices in markets close to fish farms.

Average relative effects of anomalies on prices are estimated taking logs for prices (not reported). Anomalies may have the major effect in seabass, decreasing prices by 38.2 %. For meagre, average price reductions due to anomalies are 18.2 %. The lower effect generated by anomaly events seemed to be in seabream by 13.3 % price decrement in average terms along the analyzed time series.

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Table 1. Regression model for seabass, seabream and meagre

Dep. variable Price (€)	**Seabass** Coefficient (Robust St. Error)	**Seabream** Coefficient (Robust St. Error)	**Meagre** Coefficient (Robust St. Error)
Landings (kg)	-0.001 ***	-0.001 **	-0.001 ***
	(0.000)	(0.000)	(0.000)
Anomaly (unit)	-4.180 ***	-1.906 ***	-2.178 ***
	(0.582)	(0.368)	(0.197)
Oil Price (€)	0.048 ***	0.013 ***	
	(0.006)	(0.005)	
Vessel Number (unit)	0.104 **	0.080 ***	
	(0.040)	(0.023)	
Distance (km)	0.228 ***		0.078 ***
	(0.036)		(0.016)
S1	-2.111 ***	1.970 ***	0.607 **
	(0.370)	(0.351)	(0.228)
S2	-0.340	3.115 ***	0.755 ***
	(0.400)	(0.375)	(0.200)
S3	2.891 ***	4.960 ***	1.297 ***
	(0.390)	(0.502)	(0.168)
Constant	4.542	5.963 ***	5.586 ***
	(2.814)	(1.219)	(0.412)
Nº observations	34322	56745	13117
F-test	104.592	47.607	46.156
(p-value)	(0.000)	(0.000)	(0.000)
R-squared	0.206	0.150	0.139
Mean VIF	3.39	3.42	2.11

The p-values at 0.001, 0.05** and 0.01*** significance level. VIF = Variance Inflation Factor. Source: Author's own elaboration*

Conclusion

This research detects significant effects of aquaculture on prices of three wild fishes (i.e., seabass, seabream and meagre), as long as the activity induces anomalies in fish landings. However, the quantitative effects here presented must be interpreted with caution, as market specific data for controlling other price determinants was lacking. Indeed, fish escape impact on market prices is a topic which is worth to research further in order to evaluate the total economic impact of these events on the sector as well as the consumers.

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DIGIHEART - MACHINE LEARNING FOR PREDICTING AND PREVENTING PRODUCTION LOSS IN AQUACULTURE

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Introduction

The annual mortality rate for farmed salmonid fish in Norway has been relatively stable in recent years, reaching up to 20% from sea transfer to slaughter (Sommerset *et al* 2021). Fish mortality poses a serious obstacle to sustainability, ethics, and economy of the Nordic aquaculture industry. Hence, reducing mortality should be considered a top priority. Currently, the causes of stress-related mortality are uncertain, but an increasing body of evidence suggests that the majority of farmed fish die from cardiac failure associated with deviating cardiac morphology or cardiac disease (Poppe *et al* 2003; Frisk *et al* 2020). Throughout production cycles, fish mortality is a key indicator to measure fish health and -welfare, production performance and efficiency.

Since cardiac morphology is a novel and promising indicator of mortality-risk, there is an urgent need to develop tools for on-site evaluation of cardiac morphology and -health, efficiently and standardized. Image analysis of hearts is typically performed by manually segmenting and measuring shapes and angles of the heart, which is a tedious and time-consuming process that require training and alignment of personnel to make consistent measurements. However, this process can be automated using machine learning models for computer vision. By building consensus amongst experts for the annotations the models are trained on, the automated process will allow more objective and accurate morphological scoring and increase capacity and accessibility for less experienced and remote personnel.

Materials and methods

In this study, we developed deep learning models for automation of cardiac morphology measurements in farmed salmonids. A total of 679 hearts (consisting of atrium, ventricle and bulbus arteriosus) from farmed Atlantic salmon (*Salmo salar* L.) were photographed in two projections (i.e., dorsoventral and lateral left). A selection of the images, including 555 and 615 images in the dorsoventral and lateral left projection, respectively, were manually annotated by a team of academic and industrial experts and used to train the computer vision model.

Moreover, to increase the quality of the model an annotation guideline was created that serves several purposes, ensuring that the relevant features are captured and that those features could be reliably produced both by human annotators and machine learning models. The model performance was evaluated qualitatively by a panel of experts to see if the automatically measured result agreed with their expectations.

Results

The trained computer vision model, based on deep learning, successfully segmented salmon hearts and detected points of interest so that the computation of geometric properties could be automated. In the vast majority of cases, the measurements were in agreement with experts' expectation. The intended workflow is that the expert will use the computer vision model as decision support and check all the automated annotations. Hence, even if a minority of pictures need to be annotated manually (in cases where the model output deviates from what the experts judge as reasonable), the model can save significant amounts of annotation time.

Based on the output from this machine learning model, the aquaculture ecosystem will now be able to use standardized data to improve diagnostic accuracy. In combination, this will enable improved forecasting of disease progression and mortality events, hence enabling more informed decisions and implementation of preventive measures. Ultimately, this will contribute to mitigated production losses, reduced environmental footprint and improved fish welfare in the aquaculture industry.

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Acknowledgments

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CHARACTERISATION OF THE GUT MICROBIOTA OF FLATHEAD GREY MULLET *Mugil cephalus* FED BACTERIAL SINGLE CELL PROTEIN

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Introduction

Flathead grey mullet (*Mugil cephalus*) is a promising omnivorous fish species for a sustainable Mediterranean aquaculture diversification due to the low dietary requirement of nutrients and the high market price of its roe (bottarga) (Solovyev and Gisbert, 2022)two and ten meals per day (R1, R2 and R3, respectively. Bacterial single cell ingredients produced by fermenting industry by-products represent a sustainable alternative of soybean meal (SBM) and fishmeal (FM) (Glencross et al., 2020). The application of bacterial single cell protein (SCP) ingredient were assessed on the blood biochemistry parameters and the gut microbiota of flathead grey mullet. Gut microbiota structure in farming condition is often associated with nutrients absorption and fish health. To the best of our knowledge this is the first characterisation of grey mullet gut microbiota diversity and structure under captive conditions and fed different diets.

Materials and Methods

Two isonitrogenous and isoenergetic experimental diets containing different SCP and SBM inclusion (SCP0, 0% SCP and 30% SBM; SCP10, 10% SCP and 9% SBM) were tested on triplicated groups of 45 fish (initial weight: 67.9 g) reared in a RAS over a period of 113 days. SCP ingredient consisted of a bulk of dried bacterial (*Corynebacterium glutamicum*) single cell (73.5 % crude protein). Feed was provided to apparent satiation on a 6 hours meal once a day. Somatometric indices, blood metabolic parameters and gut microbiota (GM) (MiSeq Illumina protocol, targeting V3-V4 regions of 16S rRNA gene) were assessed on 12 fish per treatment. Differences among treatments were considered significant at $P < 0.05$.

Results and Discussion

At the end of the trial, no significant diet effect were detected on somatometric indices. The levels of glucose, creatine, Ca^{+2}, Fe, K^+ and the ratio Na / K were found significantly higher in the fish fed SCP0 compared to SCP10 ($P < 0.05$). No significant differences between the treatments were observed for the other blood metabolic parameters considered. With regards to the gut microbiota, the most abundant phyla found in grey mullet gut were Fusobacteria and Proteobacteria (shown in Fig. 1). These two taxa represent about 87% of the whole GM in fish fed SCP0 where Fusobacteria were more abundant, while 83% in fish fed SCP10 where Proteobacteria were more abundant, although no significant differences were detected. The phyla Actinobacteria and Firmicutes seems to be higher in fish fed SCP10 ($P = 0.1210$ and $P = 0.0592$ respectively). Actinobacteria represented by *Corynebacterium* genus agreed with the dietary inclusion of bacterial SCP from *C. glutamicum*, while Firmicutes, especially lactic acid bacteria, have been associated with a beneficial effect and a healthy gut ecosystem. With a focus on the class level, specific compositional differences, although non-significant, where detected in fish fed SCP10 which present high abundance of bacilli ($P = 0.1702$) and clostridia ($P = 0.0621$). These two classes of bacteria include some promising probiotics which have been shown to be beneficial for the immune response and disease resistance in cultured fish (Guo et al., 2020; Kuebutornye et al., 2019). Overall in both groups, the more abundant genus was *Cetobacterium,* which have been found to be dominant in the gut of many freshwater species and have a positive impact on the digestion and metabolism efficiency of the host. Finally (sown in Fig. 2), no difference in the gut ecosystem diversity were detected at phylum and genus level between the two groups, however at species level there is a tendency for higher diversity in fish fed SCP10 (α-diversity; $P = 0.1280$).

Conclusion

Bacterial SCPs are promising circular ingredients which also present a probiotic effect. As such, further research is needed to better understand their application on grey mullet diet. Moreover, data from the gut microbiota contribute to the deep characterisation of the taxonomic diversity of the grey mullet gut bacterial community which is essential for understanding the dynamics of the diet-host-gut microbiota axis.

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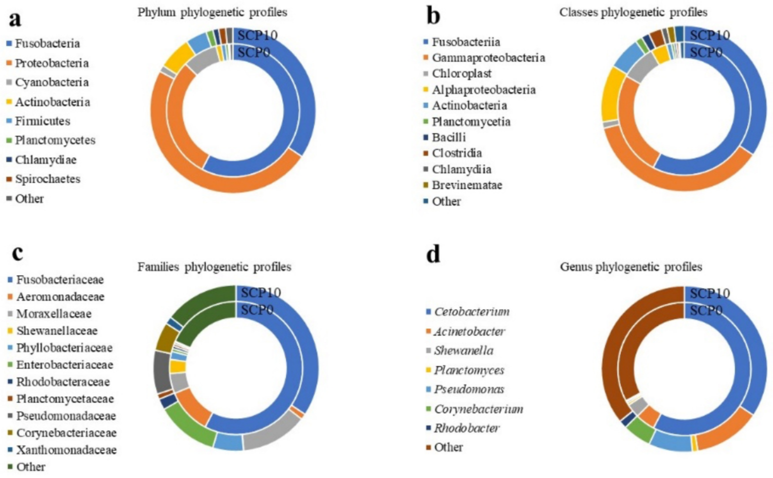

Figure 1. Average phylogenetic profiles of grey mullet gut microbiota at phylum (a), classes (b), families (c) and genus (d) level.

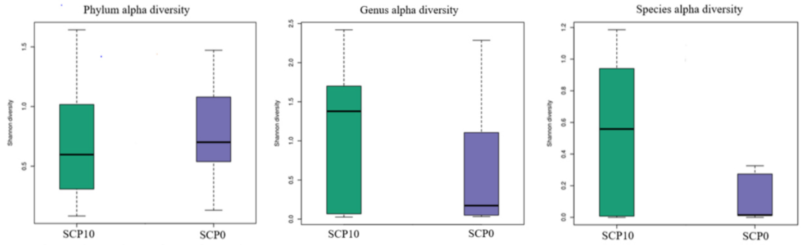

Figure 2. Shannon alpha diversity of grey mullet gut microbiota at phylum (left), genus (centre) and species (right) level.

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BLOOD TRANSCRIPTOMICS AND MACHINE LEARNING, A NON-LETHAL APPROACH FOR FISH RESEARCH: A CASE STUDY ON SALINITY HABITAT HISTORY OF EUROPEAN EEL (*Anguilla anguilla*)

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Introduction

Reducing the number of lethal analyses is fundamental in fish research, therefore alternative non-lethal approaches need exploration. For this purpose, the investigation of blood transcriptomics is of great interest. Blood transports molecules throughout the body and can be sampled even multiple times without sacrificing of the animal. RNA is relatively stable, and small quantities are enough for parallel massive gene expression profiling through high throughput techniques (i.e. RNA-seq). In fish, blood transcriptomics is still underutilized, although the few studies available show how it can be informative in a wide range of assessments. In this work, we focus on the European eel *Anguilla anguilla*, which is a critically endangered species with a key aquaculture interest. In nature, yellow eels may live in different salinity habitats (i.e., fresh-, and seawater), and this information is typically obtained through the analysis of otolith microchemistry, which requires the sacrifice of the fish. We combined blood transcriptomics and machine learning (i.e., random forest) to test the ability of transcriptomic blood-based analysis to predict salinity habitat history guided by their otolith-based classification.

Material and methods

A total of 60 eels were caught between July and August 2020, in different freshwater, brackish water and seawater sites in Norway. From each eel, otoliths and 600μl of whole blood were collected. Otolith thin sections were analysed for ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr and ^{137}Ba to classify eels into three different salinity habitat behaviours: freshwater resident (FWR), seawater resident (SWR) and inter-habitat shifter (IHS; i.e. eel that switched one or multiple times between freshwater and seawater habitats).

RNA-seq from RNA of whole blood was performed, generating ~43M of paired-end reads for each sample. Reads were mapped against the European eel reference genome with tophat2 [1]. Read count, the step to determine gene expression on the ~31,000 eel genes, was performed with htseq-count [2]. Differential expression (DE) was performed with Deseq2 [3] comparing SWR and FWR eels to assess the pool of genes to use for machine learning. Genes with adjp-value <0.05 were considered as significantly DE.

Random forest with the DE genes was run over normalized gene expression data, using the R package randomForest (www.stat.berkeley.edu/~breiman/RandomForests/). For this analysis, we considered the whole sample set of eels, which had been previously classified into FWR, SWR and IHS according to their otolith microchemistry. From the first round of analysis with all the DE genes, a further reduction was performed based on Mean Decrease in the Gini Index to consider only genes with the highest classification power. RF was then repeated with the top 150, 100, 50 or 30 genes with the highest Gini values.

Results and Discussion

The DE analysis between FWR and SWR detected 3,451 DE genes. Figure 1 shows the principal component analysis of each eel based on the blood gene expression of these DE genes and labelled according to the otolith microchemistry analysis. FWR and SWR eels are well separated on the plot, with IHS samples between the two groups.

Random forest performed using the complete set of DE genes, classified eels based on their salinity habitat with an average error of 21.07%, with mis-assignments mostly for FWR and IHS (Table 1). The further subset of genes selected, considering the Mean Decrease in the Gini Index value improved the overall results, especially in the correct assignment of FWR individuals, reaching a minimum of 6.9% using 50 or 30 genes, where only four misplaced animals belonging to IHS were misclassified (Table 1).

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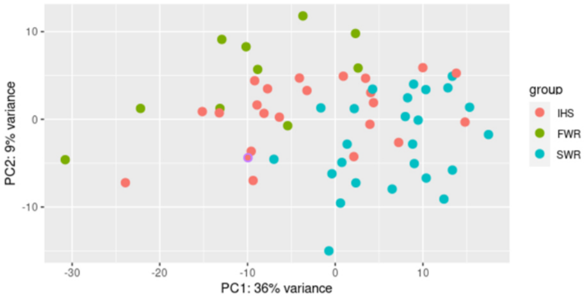

Figure 1. Principal component analysis of the DE gene expression values measured in each eel blood sample. Different colors identify the various habitat histories (i.e., IHS, FWR, SWR) of eels determined by otolith microchemistry analysis.

N. genes	**All DE**	**150**	**100**	**50**	**30**
% overall assigment error	18.97	8.62	8.62	6.9	6.9
% correctly assigned FWR	80	100	100	100	100
% correctly assigned SWR	96	100	100	100	100
% correctly assigned IHS	67	80	80	84	84

Table 1. Random forest overall classification error for each of the gene sets and the details in relation to the three otolith-based classes

Conclusions

The combination of random forest and blood transcriptomic profiling allowed the assessment of the salinity habitat history of European eels with high accuracy, showing potential to replace its lethal assessment. This approach is promising with respect to replacing or reducing other lethal approaches in fish aquaculture and monitoring, and suitable for both controlled and in-field experiments, as well as for longitudinal studies.

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MODELLING CO2 SEQUESTRATION POTENTIAL OF MUSSEL FARMS ACROSS THE MEDITERRANEAN SEA

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Introduction

In the Mediterranean sea Mussels represent 11% of the share of aquaculture production by weight, being the fourth most cultivated species, in terms of value this translates to 3% (Carvalho & Guillen, 2021). Shellfish are increasingly being looked at as sustainable food sources that provide additional ecosystem services, such as water filtration and carbon sequestration. Quantifying some of these ecosystem services (i.e. their as carbon sinks) is a necessary step in order to manage and regulate additional finances to the sector (e.g. via carbon credits). In this study we focused the question on understanding carbon fluxes across Mussel cultivation sites in the whole Mediterranean Sea basin. To avoid arbitrary choices on what fluxes to include, all aspects were considered: the sequestration in soft tissue and shell, the emission of CO2 associated with calcification and the CO2 respired.

Methods

A bioenergetic model based on the Scope for Growth theory (Brigolin et al., 2009) was employed and adapted following Bertolini et al. (2021)their role as CO2 sinks or sources is still debated. To quantify it, shell accretion dynamics should be accounted for. However, the shell component is usually calculated with allometric scaling, in most bivalve models. With this study, shell accretion was added to a bioenergetic model of the Manilla clam (Ruditapes philippinarum to include shell formation. Twelve stations were chosen to span the whole Mediterranean basin in which mussel cultivation is known to take place. Model forcings included sea surface temperature, salinity, chloprophyll-a and particulate organic carbon concentration, ph, and alkalinity. These were acquired for these sites through CMEMS website (https://marine.copernicus.eu) and google earth engine (https://developers.google.com/earth-engine/datasets/catalog/NASA_OCEANDATA_MODIS-Aqua_L3SMI) , and included both earth observation and operational oceanography products. Time series considered spanned over 20 years, starting from 2000 up to 2019. Sea surface temperature data were at a daily frequency, while time series of the other variables had a monthly frequency, and were therefore interpolated by means of a spline algorithm to comply with the daily time step required by the individual model.

Results

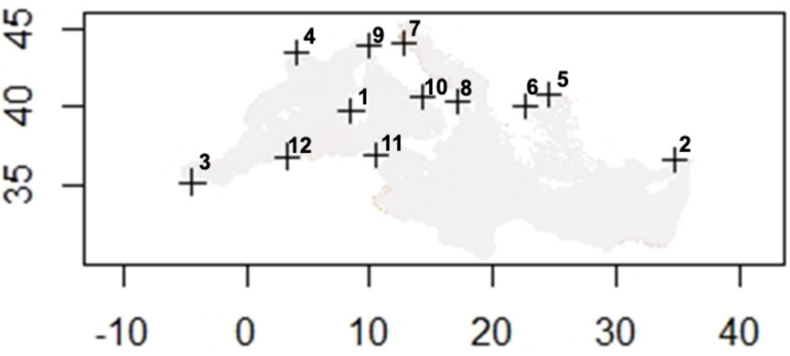

Fig.1 Location of the 12 stations investigated within this work.

When only the two components of shell formation (sequestered minus emitted during the calcification process) are considered, mussels are overall sinks of circa 0.5g CO_{2eq} per mussel. When all components are considered together, mussels can be a sink of c.ca 2g $CO_{2eq.}$

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Discussion and conclusion

The extent of sequestration depends upon site-specific mussel productivity, with lower values in areas where mussels are smaller (e.g. stations 2 and 7). Furthermore, the inclusions of different aspects in the carbon budget will depend upon the needs, end goals and management practices within farms (e.g. selling of whole vs processed product, fate of shells…), therefore this approach can be used as an useful tool for budget calculations also as support for financial actions (e.g. carbon credits).

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WHOLE GENOME SEQUENCING DATA PROVIDE INFORMATION ON SIGNATURE OF SELECTION IN CULTIVATED GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Seabream selective breeding is an increasing and key aspect in improving Mediterranean aquaculture, especially in Italy, where this is one of the most farmed species. Despite the recent improvement in genomic tools, breeding programs including genetic or genomic controls are uncommon. Uncontrolled mating could lead to inbreeding increase and to loss of genetic variability in specific genomic regions, so potentially affecting future breeding plans. In this work, we applied a comparative DNA-pooling sequencing approach to compare five pools of juveniles from three different families (broodstock pools) in an Italian hatchery.

Materials and methods

Juveniles were sampled from the same commercial hatchery. These samples were offspring from three different broodstock nuclei (nucleus A, B and C). These samples were used to create 5 different equimolar DNA pools of about 30 animals each: 3 pools for nucleus A (A1, A2, A3), and one for each nucleus B and C. Whole-genome re-sequencing of the five DNA pools was done using Illumina paired-end sequencing. In addition, Pool-seq data from several farmed and wild populations across Europe containing approx. 30 animals each were considered [1]. For each pool, reads were aligned to the gilthead seabream Saurata_v1 reference genome (accession no. GCA_900880675.1) using BWA 0.7.12 with the *mem* function [2] and standard options. Variant calling on the mapped reads was performed with the CRISP software using default parameters [3]. Only bi-allelic single nucleotide polymorphisms (SNPs) with quality >100 and depth ≥40 among the groups were considered. Pairwise FST between juvenile DNA pools was calculated for each SNP located on assembled chromosomes and then averaged on overlapping 500 kbp windows [4].

Results and discussion

The variant calling produced approximately 11M of SNPs. Principal Component Analysis (PCA) detected a similarity of the juvenile pools from the same family (A1, A2 and A) that is close to other farmed pools considered, while the remaining two pools were highly distinct from the rest of the group (Figure 1a) and different from each other (Figure 1b). Therefore, A1, A2 and A3 were considered as one pool for the subsequent FST analyses.

FST analyses detected regions in 11 out of the 29 chromosomes. Some divergences correspond to regions of fixed alleles for pools B and C. The highest number of regions were found on chromosome 6, with distinct patterns for each comparison (Figure 2). This chromosome has been suggested as containing candidate gene(s) for lack of pigmentation in pool A (Bertolini et al. 2021). In these regions, loss of heterozygosity has been detected for pools B and C in correspondence to 22 and 26Mb for pool B and 37 Mb for pools B and C (Figure 2). These regions contain genes with binding and catalytic activities involved in several metabolic processes.

Conclusions

The results of this study provide an overview of possible effects of localized loss of genetic variability in some of the cultivated population and suggest the need to implement genetic control of broodstock in order to control the genomic structure of offspring.

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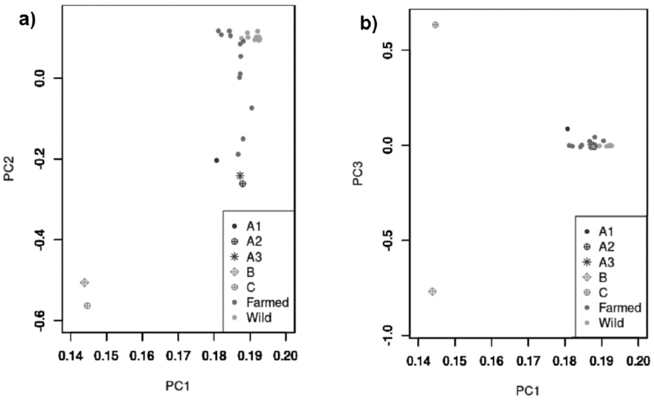

Figure 1. PCA considering the filtered SNPs.

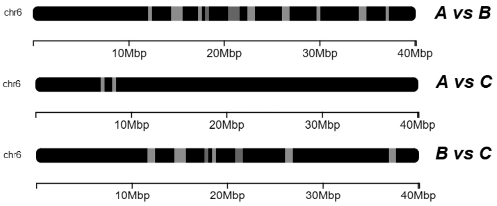

Figure 2. Regions of higher divergences on chromosome 6 for the different comparisons. Regions with the highest FST values are marked in green, regions with the highest FST and almost fixed for pool B or C are marked in red.

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GENETIC PARAMETERS OF EDIBLE YIELDS AND THEIR NON-LETHAL PREDICTORS IN TURBOT (*Scophthalmus maximus*)

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Introduction

Turbot is the most flatfish species produced in the world, China accounting for more than 65 000 tons in 2015. Selective breeding programs emerged in the early 1990s in Spain and France. Still, few heritability and genetic correlation estimates have been reported regarding edible yields, which are lethal phenotypes to measure. Recently, Schlicht et al (2019) filleted turbots and estimated a heritability of 0.15 for fillet yield for sib-selection. At this time, there is no report of relationship between morphometric and non-lethal traits into a variable that may predict the fillet yield of candidates. The aim of our study was thus to estimate the genetic parameters of non-lethal measurements to evaluate the possibility to predict edible yields on live selection candidates in a French commercial line.

Material and Methods

The fish were derived from the France Turbot Ichtus breeding program in August 2018, using 53 sires and 13 dams mated in a partial factorial design by controlled matings, producing 87 full-sib families. Larvae were reared in common environment. Fishes were individually tagged at 666 days post hatching (dph) (~270g). 897 progenies and their parents were tissue-sampled for DNA parentage assignment using a 96 SNPs panel of markers developed in this project (Gentyane, Clermont-Ferrand, France). The turbots were reared until slaughtering at 750 g (1013 dph) without any selection. The individual body weight (BW) was recorded as well as the weights of different parts: dorsal, ventral and head with abdominal cavity. They were modeled with a linear regression with BW to obtain phenotypic and genetic parameters for residual dorsal part weight (rDW), residual ventral part weight (rVW), residual head and cavity weight (rHCW). The sum of dorsal and ventral parts was used as surrogate for the edible yield relative to BW (rDVW). Non-lethal measurements were made: an individual picture was taken as well as 3D coordinates (Microscribe G2X, Revware Inc., North Carolina) at 19 landmarks on each fish. Geometric morphometrics and computation (MorphoJ and R software) enabled to define new traits such as lengths, areas and volumes. This large number of variables (~50) was evaluated using the Regbest function (in R) to define models that predict the yields. These are the best compromises in terms of model accuracy, percentage of prediction and easy to get variables from field data (*e.g.* whole-body area, area of the head, distance from snout to operculum, etc.). Heritabilities (h^2) and genetic correlations for all traits were computed based on multivariate linear mixed animal models fitted by restricted maximum likelihood in VCE (v6, Groeneveld et al., 2008).

	pDW	pVW	pHCW	pDVW
rDW	0.47	0.49	-0.74	0.38
rVW	0.94	0.99	-0.70	1.00
rHCW	-0.91	-0.77	0.90	-0.92
rDVW	0.76	0.82	-0.79	0.82
pDW	**0.30 (0.11)**	0.49	-0.78	0.80
pVW	*0.49*	**0.31 (0.11)**	-0.42	0.82
pHCW	*-0.70*	*-0.57*	**0.29 (0.11)**	-0.45
pDVW	*0.65*	*0.80*	*-0.62*	**0.31 (0.10)**

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Results and Discussion

The assignment rate of the progeny to their parents using APIS software (Griot et al., 2019) was 78% (the reason for this lower than expected result is still being explored), the dataset comprising then 707 turbots from 8 dams and 29 sires (56 full-sib families). The edible yields (expressed as residuals) had low to intermediate h^2: 0.12 ± 0.06 for the dorsal part, 0.16 ± 0.07 for the ventral part, 0.23 ± 0.10 for the head and cavity part, and 0.28 ± 0.10 for the sum of edible parts (Schlicht et al 2019 get a heritability of 0.15 ± 0.03 in the range of what we estimated). Their predictors had higher h^2 (~0.30 ± 0.11) (Table 1) but did not differ significantly. Phenotypically, the predictors predicted only a limited proportion of the real yields: 8% for the dorsal part, 9% for the ventral part, 15% for the head and cavity, and 15% for the edible parts. However, the genetic correlations between the real yields and their predictors were strong: 0.47 for the dorsal, 0.82 for the edible parts, 0.90 for the head and cavity part, and up to 0.99 for the ventral part.

Table 1: Heritability (and standard error) of the yield predictors (pDW, pVW, pHCW, pDVW) (in bold); genetic correlations and phenotypic correlations (in italics) between the predictors and the real yields (rDW, rVW, rHCW, rDVW).

The predictors defined in turbot in this study explained a lower proportion of the real yields to predict compared to previous studies in round fish such as rainbow trout (37% for carcass yield, Haffray et al., 2013), common carp (63% for headless carcass yield, Prchal et al., 2018), European sea bass (27% to 38%, Vandeputte et al., 2017). However, the genetic correlations were much higher in our study. And because the predictors are heritable traits, the selection on yield predictors in turbot may be at least as effective as relying on family data acquired by slaughtering the sibs to know the real yields.

Conclusion

Predictors are moderately heritable and positively genetically correlated with the real yield they predict. This means that indirect predictors can be used to index candidates themselves through the use of non-invasive measurements that should enhance accuracy of breeding evaluation, a work in progress.

Acknowledgement

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RECENT ADVANCES IN CHEMOSENSING MECHANISMS IN BIVALVE MOLLUSCS: MORPHOLOGICAL AND MOLECULAR INVESTIGATIONS

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Introduction:

Bivalve molluscs are notoriously filter-feeding animals, through the gills (ctenidium) are able to simultaneously hold oxygen and nourishment consisting of microplankton and particles suspended in the water[1]. Currently the mechanism by which microplastics are retained or not inside the bivalves is still unclear. In addition, in literature has been reported how these marine organisms are able to select and discriminate microplankton from microplastics (10 ÷ 15 µm) playing the role of biodepurators[2]. Moreover, some authors indicate that upon exposure of bivalves to microplastics, it is likely that microplastic can enter the pallial cavity and adhere to the mantle or gills and possibly embedded in mucus[3]. It is plausible that the gills of bivalves play a key role in the chemosensing.

In vertebrates, sensing of luminal contents by the gastrointestinal (GI) tract mucosa plays a critical role in the control of digestion, absorption, food intake and metabolism by triggering functional responses appropriate for beneficial or potentially harmful substances. In this complex chemosensing mechanism, taste receptors (TRs) ant their respective G alpha subunit (e.g. α-gustducin) are involved. In addition, the majority of these luminal chemosensors are expressed on enteroendocrine cells (EECs). In this study we showed preliminary data, obtained using immunohistochemistry (IHC), on the presence and distribution of cells expressing markers for TRs, hormones and mucins in mussel's gills.

Aims:

The aims of this study were to investigate on the possible chemosensing mechanism in the gills of molluscs by which these invertebrates discriminate nutrients and non-nutrients (included microplastics) substances present in marine environment.

Material and methods:

IHC was performed in Niedditas mussel's gill using T1R3 (santa cruz sc-50353). We performed also IHC using the pan-neuronal marker HuC/D (invitrogen A21272), mucin 4 (invitrogen 689328A), α-Gusducin (santa cruz, sc395), cholecystokinin-8 (CCK-8, abbexa; abx090677), ghrelin (santa cruz; sc10368) and neuropeptide Y (NPY, Chemicon AB1583). Western blotting was performed on total lysate of mussel's gill using T1R3 antibody.

Results:

By means of IHC, we showed that the T1R3 (sweet and umami receptor) as well as α-gustducin are expressed in the mussel's gill. T1R3 is a heterodimer involved in a sweet, umami and L-amino acids perception. Interestingly, the expression for T1R3 was also confirmed *via* western blotting. Furthermore, mussel's gill expresses orexigenic peptides such as NPY, ghrelin and the CCK considered a potent anorexigenic hormone. In addition, the mussel gills exhibited immunoreactivity for mucin-4 (one of the most expressed glycoproteins in mammal's mucus). Finally, we also observed immunoreactive cells for the pan neuronal marker HuC/D in the gills.

Conclusions:

Collectively, the data demonstrate that the mussel's gill expressed T1R3 and α-gustducin as well as some hormones with an orexigenic / anorexigenic action. The majority of these immunoreactive cells exhibited an elongated shape (generally flask-shaped). These histological features, together with their surface position, suggests that such cellular elements could be of an endocrine nature (similar to mammals "open type" enteroendocrine cells). The presence of the pan neuronal marker HuC/D might suggest that mussels may have a chemosensory system similar to the other invertebrates. As previously suggested by several authors, these cells could be typical bipolar neurons, with cilia projecting towards the free surface (seat of the chemosensing) and with an axon at the proximal end that projects into the central nervous system. This initial finding provides a basis to further investigate the full spectrum of chemosensing mechanisms in this molluscs as mechanism for the food-intake discrimination.

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EFFECT OF ESSENTIAL OILS AND THEIR BLENDS ON FISH PATHOGENS

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Introduction

In aquaculture the evidence concerning antimicrobial resistance has expanded and evidenced that some antimicrobials are only partially effective against some fish pathogens (Miller & Harbottle, 2018). The growing impact of resistant bacteria on human and animal health, as well as on the environment, indicates the need for supporting agents to antibiotics, such as natural compounds of plant origin. The application of essential oils opens a perspective for an environmentally friendly and sustainable control of diseases caused by pathogens in aquaculture.

Material and methods

Using minimum inhibitory concentration (MIC) assays with microdilution broth technique, we investigated the antimicrobial activity of eleven essential oils (EOs) and two blends (GL mix and BR mix) against pathogenic bacteria that could cause infections and disease outbreaks in fish farms. The pure essential oils were obtained from the species *Origanum vulgare* (Ov), *Melaleuca alternifolia*, *Melaleuca leucadendron* (Ml), *Eucalyptus globulus*, *Mentha x piperita*, *Lavandula angustifolia* (La), *Lavandula hybrida* (Lh), *Cinnamonum zeylanicum* (Cz), *Rosmarinus officinalis*, *Citrus limon* (Cl), *Thymus vulgaris* (Tv)). These oils and blends were all provided by APA-CT (Forlì, Italy) and their chemical composition was analyzed by gas chromatograph coupled with mass spectrometer (GC-MS). Inhibitory effects of EOs were tested against reference and field strains of fish pathogenic bacteria: *Aeromonas salmonicida* ATCC 33658, , *Yersinia ruckeri* ITT 100/16, *Photobacterium damselae* subsp. *piscicida* ITT 210/B/21, *Vibrio harveyi* ITT 11/16, and againstb*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 8739 as controls.

Antibacterial susceptibility testing was performed according to EUCAST for broth microdilution assays (2017). The effectiveness of each essential oil and blend was tested at twelve different concentrations, ranging from 3.125% (31.25 mL/L) to 0.0015% (0.015 mL/L) using Alkamus (Solvay Solutions, Milano) 3.75% (v/v) as an emulsifying agent. For most bacteria tested, Mueller Hinton broth (supplemented with 1.5% NaCl for *Vibrio harveyi*) was used as the medium. For *Photobacterium damselae* subsp. *piscicida* Tryptic Soy Broth (TSB) with the addition of 1.5% of NaCl was used. Each assay was carried out in triplicate. A positive control to verify the growth of each bacterium and a negative control containing only the tested concentrations of EOs were run concurrently with the test item(s).

The bacterial suspensions were diluted with sterile saline solution to the concentration of 1.0×10^5 CFU/mL. To avoid loss of moisture and volatile compounds of essential oils during incubation each plate was sealed with sterile, self-adhesive aluminum foil. The microplates were incubated at 37 °C or at 25 °C, depending on the optimal bacterial temperature. After 24h incubation time, the optical density of each well was measured using a 620 nm wavelength Microplate Reader (Life Technologies) and compared to a blank one (broth medium with diluted essential oil) and positive control. Both Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined (EUCAST, 2003, 2017) The MIC is defined as the lowest concentration that completely inhibits the growth of the specific organism, compared with the growth of the positive control; the MBC is defined as the lowest concentration resulting in the death of 99.9% or more of the initial inoculum. To determine MBCs, 10 μL of broth was taken from the well showing no microbial growth, inoculated on growth agar medium and incubated at requested conditions; after the specific incubation time required for each strain, CFU/mL were counted to assess cell viability.

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Results and Discussion

Complete suppression of most strains was obtained for most of the tested EOs and blends, except for *Pseudomonas aeruginosa* for which MIC was obtained only for Cz. In particular, the EOs with high antibacterial activity were Ov, Cz, Tv showing an average MIC value of 0.050 % ± 0.06, whereas La, Lh, Ml and Cl showed the lowest antibacterial activity with an average MIC value of 1.9% ± 1.65. The blends showed an intermediate MIC values between the highest and lowest EO MIC values. Obtained preliminary results allow to demonstrate high antibacterial activity of most of the tested EOs on some fish pathogens and their potential applications in the control of fish bacterial infections. Next steps will be aimed to extend MIC assays to other bacterial fish pathogens and to carry out in vivo studies to verify specific EO use in the context of gastrointestinal dysbiosis and pathogenesis.

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THE ITALIAN SOCIETY FOR APPLIED SHELLFISH RESEARCH (SIRAM) AND MAIN RESEARCH TOPICS OVER THE LAST 10 YEARS

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Introduction

The Italian Society for Applied Shellfish Research (SIRAM) is a non-profit scientific society born in 2011 to promote research studies for the sustainable development of shellfish farming, tackling issues faced by the sector and sharing knowledge and insights to its stakeholders. As such, it fosters dialogue among scientists, industry and regulators to align on a common view of the shellfish sector and its challenges.

SIRAM major areas of interest include quality and food safety, physiology, pathology and genetics, farming techniques, environmental interactions and ecosystem services, the technological innovations and the application of the legislation and recommendations. SIRAM members belong to academic and research organizations, public health institutes for animal health and food safety and competent authorities, independent technicians and consultants, shellfish farmers and producer associations, which participate on research dissemination and exchanges of knowledge and best practices.

This work presents an overview on the main research topics addressed during the annual conferences organized by SIRAM over the past 10 years.

Materials and methods

Scientific papers submitted at the annual SIRAM conferences from 2012 to 2021 were categorized by major topics (i.e. food safety, animal health, environmental issues and farming techniques) and subtopics (i.e. research and innovation, chemical or microbiological contamination of the product, marine biotoxins, diseases, product valorization, analytical techniques, shellfish genetics, legislation aspects, depuration process, information systems, climate changes, zoning, waste, alien species).

Results and Discussion

From 2012 to 2021, 210 papers have been presented to the SIRAM annual conferences, with a mean of 23 per year. Food safety is the most addressed topic (54%), followed by animal health (22%), environmental issues (12%) and farming techniques (12%).

Ranking the papers by subtopic, the most represented are research and innovation (17%), legislation aspects (13%), microbiological contamination of shellfish (12%) and genetics (12%), followed by diseases (9%), marine biotoxins (8%), chemical contamination (8%) and the other research subtopics (21%) as product valorization, depuration process, analytical techniques, information systems, climate changes, zoning, waste and alien species.

Emerging challenges include adaptation to climate change, identification of allocated zones for aquaculture, more eco-friendly production methods, precision farming and valorization of ecosystem services provided by shellfish farming.

In this contest, SIRAM will continue to promote applied research acting as a bridge between industry sector, the technical-scientific community and decision-makers for the sustainability of the national seafood system and ecosystem conservation.

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DEVELOPMENT OF PCR MOLECULAR ASSAYS FOR THE DETECTION AND QUANTIFICATION OF COMMON BACTERIAL PATHOGENS OF FISH IN THE MEDITERRANEAN AQUACULTURE

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Introduction

The pathogenic bacteria *Vibrio harveyi*, *Vibrio anguillarum*, *Vibrio alginolyticus* and *Photobacterium damselae* (subs p*piscicida* &*damselae*) mainly concern fish farms, causing significant economic losses (Montánchez et al. 2020).

In recent years, molecular PCR tests have been developed for their specific detection (Conejero et al. 2003, Fukui et al. 2007, Pang et al. 2006, Hong et al. 2007, Zhou et al. 2007, Osorio et al. 2000 , Jing-Jing et al. 2011).

This study aimed to develop new rapid molecular methods using Real-Time PCR for the specific detection and quantification of the aforementioned bacteria.

Materials and Methods

Strains of the target bacteria that had been isolated and identified in the past were used as positive controls. Furthermore, bacterial strains isolated from aquaculture fish were used as samples.

Bacterial DNA was extracted using PureLink Genomic DNA kit (Invitrogen, ThermoFisher Scientific). Their identification was carried out with the help of specific primers from the literature or optimized (Table I). The protocols used were optimized for every target gene in order to apply the method of Real-Time PCR.

For the identification of *V. harveyi*, a Real-Time PCR method was developed slightly modifying the primers of Pang et al. 2006 (Table 1).

The identification of *V. alginolyticus*was accomplished using the upstream primer of Jing-jing et al. 2011 and a new downstream primer was designed.

For the identification of *Ph. damselae R*eal-Time PCR was developed using the downstream primer of Osorio et al. 2000 and a new upstream primer was designed.

Results

The sensitivity of the developed Real-Time PCR for the specific detection of *V. harve*yi was tested using a quantified strain of *Vibrio harveyi* and found to be able to reach 4 copies. Moreover, the developed method showed 93.6% efficiency. With this reaction, 70 bacterial strains of the *V. harveyi* species were identified, which were isolated from fish in Greek farms.

The sensitivity of the developed Real-Time PCR for the specific detection of *V. anguillarum* was tested using a quantified *V. anguillarum* strain and found to be able to reach 2 copies. Moreover, the developed method showed 100% efficiency. With this method, DNA extracts from liquid cultures of bacteria were examined and detected as Vibrio anguillarum, 2 bacterial strains.

The sensitivity of the developed Real-Time PCR for the specific detection of *V. alginolyticus* was tested using a quantified *V. alginolyticus* strain and found to be able to reach 2 copies. Moreover, the developed method shows 93.6% efficiency. With this method, DNA extracts from liquid cultures of bacteria were examined and detected as *Vibrio alginolyticus*, 2 bacterial strains.

The sensitivity of the developed Real-Time PCR for the specific detection of *Ph. damselae* was tested using a quantified strain of *Ph. damselae* and was found to be able to reach 2 copies. Moreover, the developed method shows 93.1% efficiency. With this method, DNA extracts from liquid cultures of bacteria were examined and 42 bacterial strains were detected as *Ph.damselae*. Subsequently, these strains were examined by multiplex-PCR (Osorio et al. 2000) to further differentiate them into *piscicida* (N=31) and *damselae* (N=11) subspecies.

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Target/ Method	Primers	Sequence 5' - 3'	Gene area	Product size	Reference
V.harveyi Real-time PCR	VhtoxF	**CT**GAAGCAGCACTCACCGAT	*toxR*	382	Pang *et al.* (2006)
	VhtoxR	**GACT**GGTGAAGACTCATCAGCA			
V.anguillarum Real-time PCR	VangF	TATCACTGTTGAAGAAGGTCAAGCACTG	*groESL*	195	Kim *et al.* (2010)
	VangR	CGCTTCAAGTGCAGGAAGCAG			
V. alginolyticusReal-time PCR	RpoXF	ACGCTGCTCAGGGGTGAAAGC	*rpoX*	330	Jing-jing*etal.*(2011)
	RpoXR new	**CCGCCTCTATCTGCATCTGACG**			
Ph. damselaeReal-time PCR	Phd16s F2	**GCGAAAGCGTGGGGAGCAAAC**	*16s rRNA*	550	*Osorioetal*. (2000)
	Phdam16S R	CACCTCGCGGTCTTGCTG			
Ph. damselaesubsp***.piscicida &damselae***multiplex-PCR	Phdam 16S F	GCTTGAAGAGATTCGAGT	*16s rRNA*	267	
	Phdam 16SR	CACCTCGCGGTCTTGCTG			
	Phdamurec F	TCCGGAATAGGTAAAGCGGG	*ureC*	448	
	Phdamurec R	CTTGAATATCCATCTCATCTGC			

Table 1: Primers' sequences for the detection and quantification of pathogenic bacteria.Differences of the reference primers are shown in bold. All the other primers used for real-time PCR although designed for PCR and their conditions amplified.

Discussion

The outbreak of fish diseases is considered a major threat for the development of the growing industry of fish farming. Therefore, the investigation and early detection of common fish pathogens is considered crucial in order to develop effective preventing measures in any aquaculture setting.

The molecular methods developed and optimized in the present study are fast and reliable in identifying phenotypically similar and phylogenetically related bacterial species.

Therefore, they can support Greek fish farming by helping in the early identification of the main pathogenic bacteria either in fish samples or furthermore, in environmental samples.

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INVESTIGATION OF THE CUMULATIVE NEGATIVE EFFECTS ON FAN MUSSEL, *Pinna nobilis* POPULATIONS IN GREECE, DUE TO THE PRESENCE OF *Mycobacterium* spp. AND *Haplosporidium pinnae*

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Introduction

Mortalities within the Mediterranean populations of Fan mussel, *Pinna nobilis* continues to devastate the populations of the species limiting them in few habitats alongside the Mediterranean coastline (Lattos et al., 2021a). Apart from anthropogenic activities (commercialization, food exploitation, habitat destruction) that led to population declines in the last decades, infections by Haplosporidian parasites alongside with the presence of multiple bacterial pathogens led to mass mortalities in Mediterranean Sea and placed the species in the IUCN Red list of Threatened species (Kersting et al., 2019). Amongst other bacteria, *Mycobacterium sp*. was detected in moribund individuals in Italy and Greece causing heavy lesions in the connective tissue (Carella et al., 2019; Lattos et al., 2020). Consequently, these two potentially pathogenic microorganisms are considered the most important causative agents of mortality events. Regarding the physiological effects of the presence of these two microorganisms little is known. This study aims to elucidate a part of physiology of the fan mussel and correlate the presence and the absence of the *Haplosporidium pinnae* in the collected individuals. Keeping in mind the limited number of individuals, the lack of healthy individuals in Greek coastlines and the presence of *Mycobacterium sp*. in all specimens, an assessment of physiology was implicated with the use of cell indicators of Heat shock (Hsp70 and Hsp90) and immune response (Il-6).

Materials and Methods

Pinna nobilis individuals were collected from three marine habitats in the Aegean Sea, i.e. Thermaikos Gulf, Thessaloniki Kalloni Gulf, Lesvos island and Limnos Island during 2019 period of sampling by scuba diving effort. Respectfully to the crtitical status of the species, a special permission (MEE/GDDDP89926/1117) was obtained from local authorities for the needs of the research. Specifically three samples from Thermaikos gulf, Thessaloniki, two samples from Kalloni gulf, Lesvos island and a single moribund sample from Limnos island, were used for the assessment of physiology (Lattos et al., 2020). Assessment of physiological indicators was conducted with the use of protocols for SDS-PAGE/immunoblot analysis as described in Lattos et al., (2021b). As for the localization of the proteins expression embedded tissue samples were sectioned and stained as described also in Lattos et al., (2021b). One-way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis was employed to test for significance at $p < 0.05$ (5%) level between all experimental groups examined herein (Lattos et al., 2021b).

Results

H. pinnae was histologically detected in four out of the six samples used for the study, while *Mycobacterium sp*. was also histologically detected in all samples used in this study.

Hsp70 was localized, in both cases, mostly in the epithelial cell of the digestive gland and less on the connective tissue [Figure 1(Ba,Bb)]. In contrast, Western blot analysis indicated that individuals additionally infected by *H. pinnae* exhibited higher levels of both Hsp70 and Hsp90 in all three examined tissues, compared to the non-*H. pinnae*-infected individuals, as depicted in grey shaded areas in Figure 1A. In contrast to the Hsp levels observed, analysis of Il-6 levels detected by Western blot did not reveal differences between the *H. pinnae* infected and non-*H. pinnae*-infected individuals (Figure 2A) except for the digestive gland (DG) of individuals from Thermaikos gulf, where Il-6 levels were higher in *H. pinnae* infected individuals and this was also confirmed by IHC detection of Il-6 Figure 2(Ba,Bb), which was observed in both connective and epithelial tissue, regardless of the *H. pinnae* presence.

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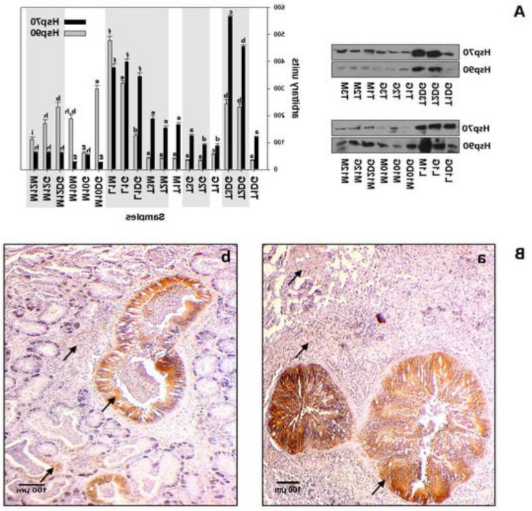

Figure 1. Hsp70 and Hsp90 levels in the digestive gland (DG), gills (G), and mantle (M) of Pinna nobilis specimens from the Kalloni Gulf (Lesvos Island) (M10, M12), from Thermaikos gulf (Thessaloniki) (T01, T02, T03), and from Limnos Island (L1). All specimens were infected with mycobacteria while grey shaded areas represent specimens additionally infected with the haplosporidian parasite.

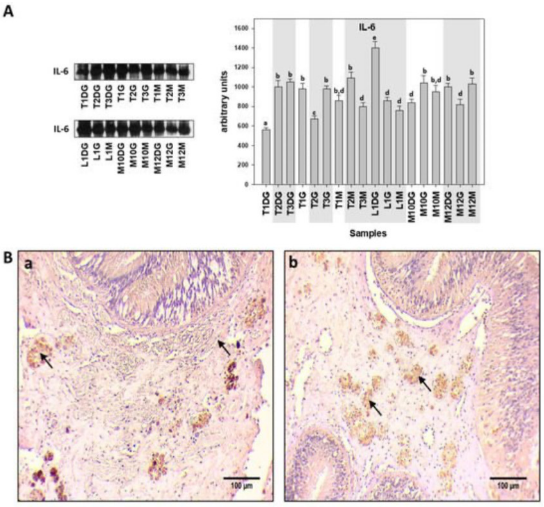

Figure 2. Il-6 levels in the digestive gland (DG), gills (G), and mantle (M) of Pinna nobilis specimens from Kalloni Gulf (Lesvos Island) (M10, M12), Thermaikos Gulf (Thessaloniki) (T01, T02, T03), and Limnos Island (L1). All specimens were infected with mycobacteria while grey shaded areas represent specimens additionally infected with the haplosporidian parasite.

Discussion

Disease etiology of mass mortality events in *P. nobilis* species is considered a complex situation, involving many microorganisms in relation to rapid changes in abiotic factors due to ongoing climate change. The results of the current research are in line with the results of Box et al. (2020), who estimated for the first time *P. nobilis* physiological performance, after the comparison of infected and uninfected animals. Individuals with both pathogens exhibited higher level of protein induction and indicated increased cellular cells, compared to individuals infected only with *Mycobacterium sp*. Hsps enable cells to adapt to various stresses and normal cellular functions are maintained by counteracting misfolded cellular proteins.

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METAGENOMIC ANALYSIS ON SKIN SAMPLES COLLECTED FROM GILTHEAD SEA BREAM (*Sparus aurata* L.) WITH RED RASH LESIONS AND NORMAL ONES

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Introduction

Red rash syndrome or red spot, is a disease encountered at an increasing level in recent years mainly in cultured gilthead sea bream (*Sparus aurata* L.). It is an important disease in many countries, like Greece, Italy and Spain, affecting different fish species (mainly sea bream, sea bass and sole). It is observed in various sizes with superficial hemorrhagic lesions on the skin and fins (Figure 1). It is usually encountered during the winter and spring months and gradually resolves on its own although symptoms can reoccur in the same cage. Its association with stress factors in different rearing conditions, has been proposed (Zrncic et al, 2020).

The fact that the disease occurs in commercial sizes of sea bream makes it one of the most important diseases in terms of economic impact. Producers in Greece have reported that during fishing, the percentage of symptomatic fish has reached almost 50% of the total population.

Empirically, it has been observed that treatment with oxytetracycline can accelerate skin healing even though no bacterial pathogen has been isolated when tissues were cultured in microbiological media.

The scope of this study was to investigate the presence of bacterial pathogens with the method of 16S rRNA amplicon metabarcoding analysis on skin samples collected from symptomatic fish and healthy ones.

Materials and Methods

During June of 2020 and 2021, skin samples were collected from 10 symptomatic and 10 healthy fish. Symptomatic fish are coded as RS1a,b,c,d, RS2,b, RS3,b, RS4 and RS5, while RSC codes correspond to control samples (normal skin) collected from the corresponding area of skin to the RS samples.

DNA was extracted using ZymoBIOMICS DNA Miniprep Kit (ZYMO RESEARCH, Irvine, CA, USA) according to the manufacturer's instruction. DNA concentration was measured in Qubit 4.0 Fluorometer with Qubit® dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA).

Bacterial diversity was determined by sequencing the V3 – V4 hypervariable regions of the 16S rRNA gene (≈ 460 bp). The protocol of Illumina "16S Metagenomic Sequencing Library Preparation" (15044223 B) was used to prepare the libraries. The libraries concentration was measured in Qubit 4.0 Fluorometer with Qubit® dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA).

The size of the DNA molecules of the libraries was estimated by capillary electrophoresis on the 5200 system Fragment Analyzer (Agilent Technologies Inc. Santa Clara, CA, USA) using the dsDNA 915 Reagent kit (35 – 5000 bp). Finally, quantitative PCR was performed to quantify the libraries (qPCR) in Rotor-Gene Q (Qiagen, Hilden, Germany) using KAPA Library Quantification Kit for Illumina Platforms (KAPA BIOSYSTEMS). Sequencing was performed on a MiSeq platform, using the MiSeq v3 reagent kit (2 × 300 cycles) (Illumina Inc. San Diego, CA, USA).

Bionformatics analysis and assessment of the bacterial communities was conducted using Qiime2 (Quantitative Insights into Microbial Ecology 2, https://qiime2.org/) v-2020.2. Raw, paired-end sequences were trimmed, quality filtered and dereplicated into unique sequences and their respective abundances by the DADA2 plug-in. Dereplicated sequences were denoised, chimeras were identified and excluded from the data and the remaining paired-end reads were merged, resulting in the creation of the final ASV table. Bacterial ASVs were taxonomically annotated using the SILVA (138) database. ASV tables containing the frequencies for each ASV in each sample of the data set, as well as their phylogenetic classification, were imported into R programming language v4.1.15, for further processing and visualization of the results. Changes in microbial composition are presented as normalized (%) relative abundance heatmaps.

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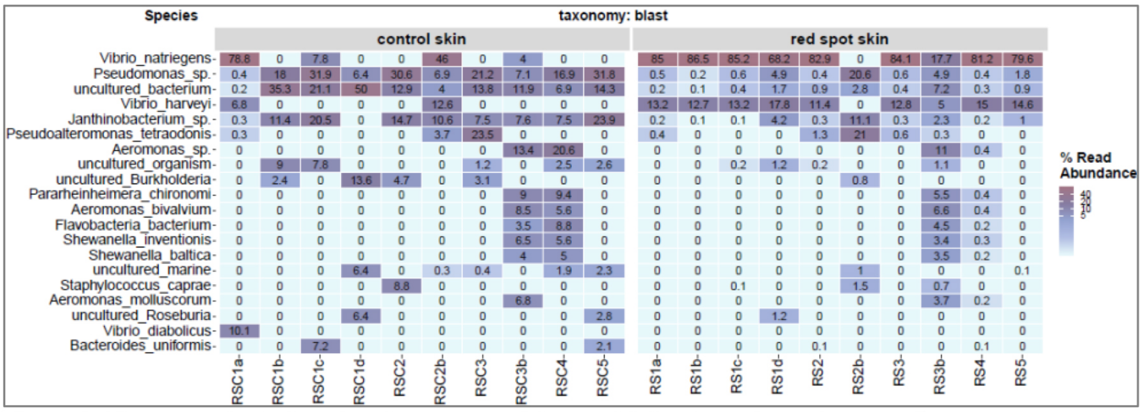
Figure 1. Red spot in gilthead sea bream.



Species	control skin										red spot skin									
	RSC1a	RSC1b	RSC1c	RSC1d	RSC2	RSC2b	RSC3	RSC3b	RSC4	RSC5	RS1a	RS1b	RS1c	RS1d	RS2	RS2b	RS3	RS3b	RS4	RS5
Vibrio_natriegens	78.8	0	7.8	0	0	46	0	4	0	0	85	86.5	85.2	68.2	82.9	0	84.1	17.7	81.2	79.6
Pseudomonas_sp.	0.4	18	31.9	6.4	30.6	6.9	21.2	7.1	16.9	31.8	0.5	0.2	0.6	4.9	0.4	20.6	0.6	4.9	0.4	1.8
uncultured_bacterium	0.2	35.3	21.1	50	12.9	4	13.8	11.9	6.9	14.3	0.2	0.1	0.4	1.7	0.9	2.8	0.4	7.2	0.3	0.9
Vibrio_harveyi	6.8	0	0	0	0	12.6	0	0	0	0	13.2	12.7	13.2	17.8	11.4	0	12.8	5	15	14.6
Janthinobacterium_sp.	0.3	11.4	20.5	0	14.7	10.6	7.5	7.6	7.5	23.9	0.2	0.1	0.1	4.2	0.3	11.1	0.3	2.3	0.2	1
Pseudoalteromonas_tetraodonis	0.3	0	0	0	0	3.7	23.5	0	0	0	0.4	0	0	0	1.3	21	0.6	0.3	0	0
Aeromonas_sp.	0	0	0	0	0	0	0	13.4	20.6	0	0	0	0	0	0	0	0	11	0.4	0
uncultured_organism	0	9	7.8	0	0	0	1.2	0	2.5	2.6	0	0	0.2	1.2	0.2	0	0	1.1	0	0
uncultured_Burkholderia	0	2.4	0	13.6	4.7	0	3.1	0	0	0	0	0	0	0	0	0.8	0	0	0	0
Pararheinheimera_chironomi	0	0	0	0	0	0	0	9	9.4	0	0	0	0	0	0	0	0	5.5	0.4	0
Aeromonas_bivalvium	0	0	0	0	0	0	0	8.5	5.6	0	0	0	0	0	0	0	0	6.6	0.4	0
Flavobacteria_bacterium	0	0	0	0	0	0	0	3.5	8.8	0	0	0	0	0	0	0	0	4.5	0.2	0
Shewanella_inventionis	0	0	0	0	0	0	0	6.5	5.6	0	0	0	0	0	0	0	0	3.4	0.3	0
Shewanella_baltica	0	0	0	0	0	0	0	4	5	0	0	0	0	0	0	0	0	3.5	0.2	0
uncultured_marine	0	0	0	6.4	0	0.3	0.4	0	1.9	2.3	0	0	0	0	0	1	0	0	0	0.1
Staphylococcus_caprae	0	0	0	0	8.8	0	0	0	0	0	0	0	0.1	0	0	1.5	0	0.7	0	0
Aeromonas_molluscorum	0	0	0	0	0	0	0	6.8	0	0	0	0	0	0	0	0	0	3.7	0.2	0
uncultured_Roseburia	0	0	0	6.4	0	0	0	0	0	2.8	0	0	0	1.2	0	0	0	0	0	0
Vibrio_diabolicus	10.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacteroides_uniformis	0	0	7.2	0	0	0	0	0	0	2.1	0	0	0	0	0.1	0	0	0	0.1	0

Figure 2. Heatmap of relative abundance rates of the top 20 dominant bacteria at species level.

Results

A total of 603,504 paired-end sequences were generated. After DADA2 analysis, 313,959 sequences remained for phylogenetic classification ranging between 828 (RSC4) and 49,263 (RS3) per sample (mean = 15,697, n = 20). These sequences were initially mapped to 2,855 unique ASVs.

In Figure 2, heatmaps showing the relative abundance percentages of the total sequences are shown. Eight out of the 10 RS samples (symptomatic fish) and 1 RSC sample are dominated by ASVs classified in the Genus Vibrio, with abundance rates > 90%. At the species level, the most dominant bacterium in the RS samples is *V. natriegens* followed by *V. harveyi*.

Discussion

Red rash syndrome is considered as the second most important disease of gilthead sea bream in the Mediterranean area, with unknown etiology (Muniesa et al., 2020). Due to its empirical treatment with oxytetracycline and yet, the failure to cultivate a pathogen with the classical microbiological methods, the goal of this study was to investigate the presence of bacterial pathogens with the method of 16S rRNA amplicon metabarcoding analysis on skin samples collected from symptomatic fish and compare them to healthy ones. Our results showed that in most of the symptomatic fish the dominant bacterium was *V. natriegens* and *V. harveyi*. *Vibrio natriegens* is widely considered a non-pathogenic bacterium found in the marine environment (Hoff et al., 2020). Furthermore, Lee et al. (2016) studying the bacterial genome, were unable to find known toxin genes or pathogenicity islands suggesting that this organism is a Biosafety Level 1 organism. Yet, there is a citation by Bi et al. (2016), isolating and identifying *V. natriegens* as a pathogen causing mortalities in cultured swimming crabs *Portunus trituberculatusin* in China. To our knowledge, this is the first time that *V. natriegens* and *V. harveyi* are being related to red rash syndrome, yet a lot of research is still needed to reach a safe result considering their correlation with the etiology of this syndrome. It could be stated that these vibrio species colonized the ulcerative lesions yet, their emergence in 9 of the 10 RS samples collected in different time periods, suggests a possible pathogenic role in the red rash syndrome.

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EXPOSED AQUACULTURE OPERATIONS. NORWEGIAN CHALLENGES AND SOLUTIONS

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Introduction

Significant parts of the ocean are today unavailable to industrial fish farming due to remoteness and exposure to harsh wind, wave, current, and ice. Conventional fish farming along the Norwegian coastline is the second most risk exposed occupation in Norway and have many challenges for the fish and the environment. Thus, conventional fish farming operates at the limits for the fish, personnel, and equipment. Although moving fish farming to more exposed areas can solve some issues, it also introduces new risks. In exposed conditions, the risks are magnified compared with conventional fish farming, making it challenging to avoid injuries, fish escapes, and mortality. Unpredictable weather and sea states lead to short operational windows and delayed or interrupted operations. Regular as well as infrequent operations are challenging. There will be postponed de-licing and longer periods of starvation for the fish.

In the Norwegian research centre EXPOSED, we have studied how exposed aquaculture operations can become robust and safe. Exposed aquaculture operations are defined as operations on aquaculture localities where work or equipment is strongly influenced by current or weather exposure. Exposed aquaculture operations share a line of challenges, that must be addressed to be able to utilize the tricky, exposed areas along the coast and at sea.

Methods

Our studies have been ongoing for seven years and embrace multiple methods. Some of the existing fish farms in Norway currently operate in exposed conditions, and these have been studied with approaches of weather data, accident statistics, technology verification results, digital twins, interviews of personnel, etc. Other methods have been development of technology or experiments with potential materials and constructions for future fish farm concepts.

Five key challenges

Through the studies we have identified five key challenges that needs to be solved to achieve robust, efficient, and safe exposed aquaculture operations.

The first challenge is risk in the human working environment. Exposed operations equal demanding working conditions that result in stress and occupational hazards. There is a need for coordinated action to reduce risks in aquaculture, involving industry and the various authorities.

Challenge two is demanding management and daily operations. Some farms have prolonged periods with ocean swell, and we have seen examples of farms that have been abandoned in periods. One needs to develop agreed competence requirements and design methods for robust technology and decision support.

Vessels serving the aquaculture farms also have demanding conditions, and this is the third challenge. The navigation alongside the farm needs to be gentle to fish that is crowded towards the surface and vulnerable to stream. There is a need for specialized vessel design and operational limits.

Challenge number four is fish welfare. Treating fish under rough environmental conditions inflict additional stress, with a potential for higher mortality. Crowding with large movements, waves, and cold air/water, represents a risk for the fish. Extreme conditions have caused mass mortality. There is a need for robust fish and adapted stocking strategies, and to understand the limitations of the fish and how to monitor its state.

The last key challenge of exposed aquaculture operations is increased risk of escaped fish. 1 of 3 escaped salmon escapes when the weather is bad. Extreme conditions also can cause structural failures. One preventive measure can be automated inspections.

Results

To solve these challenges, we have approached them from different sides. We have analysed exposure limits of fish, personnel and equipment, and found techniques to model and measure environmental loads on farms, as well as exposure degree. This has led us closer to understand how exposure and operational limits can be classified, and how exposed aquaculture operations can be robust, efficient, and safe. A selection of the solutions will be presented in the oral presentation.

PERFORMANCE EVALUATION OF THE SYSMEX XN-1000V AUTOMATED HAEMATOLOGY ANALYSER AND REFERENCE RANGES INTERVAL FOR RAINBOW TROUT BLOOD

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Introduction

Haematological parameters are valuable indicators of fish health status. Blood reflects the overall physiological status of the animal and has been widely used as a diagnostic tool to assess health status in fish in response to changes related to nutrition, water quality and pathological conditions. Haematology combined with other routine diagnostic methods can be used to identify and assess conditions that cause stress and/or diseases that affect fish production and performance. Moreover, blood samples can be collected without euthanizing the animals, providing an additional advantage for both ethical and economic reasons. Fish blood haematology analysis is currently performed mainly by manual methods as automated analysers have been scarcely validated and used in fish. Automated methods present significant advantages compared to manual techniques, offering time- and cost-effective options, eliminating operator-tied errors and, consequently, increasing repeatability and reliability of the obtained results. The present study has two main objectives: to validate the automated blood cell analyser (Sysmex XN-1000V) for its potential use with blood samples of the teleost fish *Oncorhynchus mykiss* (rainbow trout) and to establish reference values for the species since those currently available are scarce.

Material and methods

Fish used in the study were netted and anesthetized with tricaine methanesulfonate (MS-222). Blood was sampled from the caudal vein using heparinized needles (25 G) and 2 mL syringes and immediately transferred to Eppendorf tubes with heparin. Samples were analysed within 12 h after the extraction using the Sysmex XN-1000V, Ver. 3 (3.05-00), employing the settings for bird blood samples. Precision, linearity and carry-over were calculated for the results obtained from the analyser in red blood cell count (RBC), haemoglobin (HGB), haematocrit value (HCT), mean corpuscular volume (MCV), white blood cell count (WBC), and platelet count (PLT). Precision was calculated for RBC and WBC counts performed by manual method. A comparison was made between the results obtained by the analyser and the manual method for erythrocytes, leukocytes, haematocrit value and percentages of heterophils and mononuclear cells. Blood from 71 healthy fish was analysed using the Sysmex XN-1000V for the determination of the haematological reference values for rainbow trout.

Results

Precision obtained with the blood cell analyser for low-normal and high cell counts were 2.71% and 0.83% for RBC, 1.43% and 3.94% for HGB, 1.07% and 1.78% for HCT, 4.82% and 4.66% for WBC, and 0.35 and 0.12 for PLT, respectively. For MCV, heterophils (%) and mononuclear (%) the precision was only calculated with blood with normal cell counts and it was 1.39 %, 15.08 % and 2.24 % respectively. Pearson's correlation coefficient (r) for the linearity of RBC, HGB, HCT, WBC, heterophils cells (%), mononuclear cells (%) and PLTs were over 0.99 for all parameters. Carry-over for RBC and PLT were 0, but WBC was 0.57 %. Precision of the manual method was 21.7% for RBC and 17.57 % for WBC. Comparison between Sysmex XN1000V and manual method was good for most parameters with a Pearson's correlation coefficient of 0.78 for RBC, 0.87 for HCT, 0.51 for WBC, 0.68 for mononuclear cells (%) and 0.76 for heterophils (%). Reference interval range for the haematological parameters obtained from healthy rainbow trout was established (Table 1).

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Table 1. Reference values of haematological parameters of rainbow trout blood using the Sysmex XN-1000V analyser.

	n	Mean	Median	SD	Lower value	Highest value	Percentile 5%	Percentile 95%
WBC (10^3/µL)	71	22.39	21.44	10.91	2.88	51.64	3.59	43.37
Heterophils (%)	71	14.43	10.40	14.64	0.00	69.20	0.00	48.92
Mononuclear cells (%)	71	84.39	88.70	14.62	30.80	100.00	33.97	100.00
Heterophils (10^3/µL)	71	3.03	2.00	2.72	0.00	12.00	0.00	9.00
Mononuclear cells (10^3/µL)	71	20.31	18.00	11.10	2.00	49.00	2.00	41.10
RBC (10^6/µL)	71	1.03	1.04	0.18	0.65	1.55	0.66	1.32
HGB (g/dL)	71	6.06	6.00	0.99	3.90	8.80	3.97	7.66
HCT (%)	71	41.10	41.02	8.69	20.96	60.91	22.79	53.12
MCV (fL)	71	412.79	411.55	48.35	308.00	540.00	313.73	487.39
MCH (pg)	71	54.29	54.05	5.88	38.90	73.80	40.11	65.70
MCHC (g/dL)	71	13.29	13.10	1.86	8.80	18.20	8.94	16.51
RDW-SD (fL)	71	39.66	38.40	12.60	18.40	73.20	18.66	60.20
RDW-CV (%)	71	13.28	13.05	3.37	7.10	20.90	7.20	18.98
PLT (10^3/uL)	71	3.34	3.00	2.78	0.00	13.00	0.00	8.55
PCV (%)	71	36.71	36.50	7.35	21.00	50.50	21.63	48.35

Discussion

Present results show that the Sysmex XN-1000V produce more accurate and reproducible haematological data than the manual methods. Moreover, the use of the automated analyser overcome one of the main limitations of blood haematology in fish that it is the time consuming and great variability of the manual method. Reference values interval obtained using the Sysmex XN-1000V are similar to those described by the manual methods by other authors with trout blood (Zinkl et al., 1991, Jan et al., 2021) allowing its use in future haematological studies. In conclusion, with this study we validate a new tool that will contribute to the non-lethal and fast evaluation of the health status of wild and farmed fish.

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WELFARE OF CLEANER FISH IN COMMERCIAL ATLANTIC SALMON PRODUCTION

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Introduction

Lumpfish and wrasse are currently used as cleaner fish as part of the overall strategy for the prevention and control of lice in Atlantic salmon farming in Norway (Skiftesvik et al., 2013; Imsland et al., 2018). There is a growing concern and criticism about the welfare and survival of cleaner fish in net pens (Treasurer and Feledi, 2014; Imsland et al., 2020; Garcia de Leaniz et al., 2021). However, there is still a lack of studies that have monitored welfare status at several localities over a long period of time. Although operational welfare indicators (OWI) that are used to evaluate welfare in net pens are widely used, there is not yet a standardized overall welfare score that would allow for comparison of welfare data of all cleaner fish species and by different studies.

It is still unclear what factors affect lumpfish and wrasse welfare most negatively. Diseases are often pointed out as the main challenge, but other factors such as suboptimal environmental conditions can also negatively affect welfare. In addition, it is still poorly documented whether production conditions also have an effect on the welfare of cleaner fish. The lice grazing efficiency of cleaner fish is known to be affected by a combination of environmental conditions and biological factors. However, it is not known whether welfare affects lice grazing efficiency.

Material and methods

Lumpfish and wrasse welfare was monitored in 2019-2022, every month, when it was possible. Individuals were weighed with a precision of 1 g. Stomach contents were visually examined and all sea lice were counted. Each OWI was scored from 0 to 3 according to Noble et al. (2019), and thereafter squared to highlight the differences between individuals with several minor injuries and individuals with much more severe wounds. The overall welfare score was then calculated as a standardized weighed sum and categorized in four degrees of severity from good to severely reduced welfare. Timing of mechanical delousing, net change and net cleaning were registered. Seawater temperature and oxygen concentration were continuously registered by each of the sea farms.

Results

Based on our welfare model, results indicate that the welfare of lumpfish progressed negatively after transfer to net pens. While newly transferred lumpfish had good welfare, a large proportion of lumpfish had clearly reduced welfare after 6-7 months at sea. The negative development related to a combination of various factors, with mechanical delousing being the most important. Our results further indicated that reduced welfare negatively impacted cleaning efficacy. Preliminary results suggest that welfare of wrasse was already compromised by the time of transfer to sea, most likely due to challenges in nurseries. Even though welfare generally appeared to improve after transfer to sea, nutritional status seemed to worsen, indicating that feeding regimes of the sea farms were not sufficiently adapted. In addition, handling (delousing and disease treatments) seemed to have a negative impact on welfare of wrasse in net pens.

Discussion

We propose a model for calculating overall welfare from OWI that ensures a systematic and comparable collection of data on large scale monitoring studies. Assessments should not be based on average values for the entire population because it can hide variations between individuals who have very different welfare statuses (Sommerset et al., 2021). Therefore, we propose to estimate an overall welfare score calculated as a weighted sum of OWI scores. This allows to highlight the differences between individuals who have a few scratches that do not greatly reduce welfare and individuals who have much more severe wounds

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In accordance with other studies, we suggest that lumpfish should not be use in nets pens in summer, when the seawater temperatures are highest and delousing likely (Sommerset et al., 2021; Hvas et al., 2018). There is a clear need of improving nurseries production strategies to ensure a good welfare of wrasse by the time of transfer to sea. Improving feeding regimes for wrasse in nets pens also are a priority for welfare (Skiftesvik et al., 2017). Control over cleaner fish welfare and lice grazing efficiency in net pens can be achieved by introducing routine monitoring. This will make it possible to identify reduced welfare early, and thus implement preventive measures. Proper and sufficient allocation of resources is necessary to ensure good monitoring and therefore good welfare of cleaner fish in net pens.

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DAILY RHYTHMS OF CRITICAL WATER QUALITY PARAMETERS IN COMMERCIAL LAND-BASED, PARTIALLY RECIRCULATED ABALONE/*Ulva* INTEGRATED AQUACULTURE SYSTEMS, AND THE POTENTIAL FOR INCREASING RECIRCULATION RATES

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INTRODUCTION

Several land-based abalone farms in South Africa operate integrated aquaculture (IMTA) systems, growing the local abalone, *Haliotis midae*, linked to *Ulva lacinulata* grown in paddle-raceways supplied with abalone effluent. Two of these farms currently use the *Ulva* for bioremediation, enabling partial recirculation. Recirculating IMTA systems such as these have been operating commercially in South Africa for more than 15 years.

The farm studied operates routinely at 50% water recirculation, and the *Ulva* removes ammonia enabling continuous recirculation, and is also used as feed (in addition to formulated feed and harvested kelp). The farm is arranged as a series of platforms, each containing four independent modular clusters each consisting of a single *Ulva* paddle raceway (volume 300,000l, with ca. 1,000 kg of *Ulva*) linked with 42 abalone tanks (volume of each tank 8,000l, with each whole cluster containing ca. 10-15,000 kg of abalone).

A series of experiments examined water quality at the standard 50% recirculation, the potential to increase the recirculation to 75% over an extended period, and the effects of short-term 100% recirculation. The latter was designed to test the potential of the system to use full recirculation to combat occasional temporary Harmful Algal Blooms (HABs) in the intake water.

METHODS

An initial pilot experiment measured pH, ammonia (Total Ammonia Nitrogen, TAN and Free Ammonia Nitrogen, FAN), temperature and oxygen at two-hourly intervals, in triplicate clusters at the standard 50% recirculation rate over the course of a day. A second experiment compared three individual clusters operating at 50%, 75% and 100% recirculation, sampling every 6 hours for a 3-day period. The third experiment involved three clusters operating at 100% recirculation, with measurements every 2 hours, again for 3 days, with a single control cluster at 50% recirculation. Finally, a series of more detailed longer-term (ca. 1 month) hourly measurements of pH and temperature were made in both abalone tanks and *Ulva* raceways and water samples for nutrient analysis (ammonia, phosphate, nitrite and nitrate) were collected weekly at different recirculation rates.

RESULTS & DISCUSSION

At standard 50% recirculation, total ammonia nitrogen removal across the seaweed biofilters ranged from 65%-85% (mean 73%). In these conditions, water temperature increased by ca. 2°C in the system, with little difference between abalone and seaweed components. pH at 50% recirculation ranged from 8.1 in the daytime to 7.5 at night, and after mixing in the sump with 50% ambient seawater the pH similarly ranged from 8.0 to 7.5 in the abalone tank inflow. A regression analysis demonstrated a strong positive linear relationship between TAN removal and TAN load to the seaweed biofilter. There was a strong negative correlation between FAN removal and pH: as pH increased across the seaweed biofilters the level of FAN removal decreased. %TAN removal was somewhat higher in the day, while %FAN removal was greatest at night, with lower pH. Thus, whilst the *Ulva* bioremediation is beneficial from the point of view of pH and ocean acidification, the raised pH in the daytime results in some increase in FAN.

There were no significant differences between the 50% and 75% recirculation clusters for temperature, pH, TAN or FAN. At 100% recirculation, temperature was consistently 1°C higher in the daytime, and pH was around 1 unit lower throughout, although pH did not drop below 7.5 until day 3. Also at 100%, TAN and FAN increased rapidly, with TAN values ranging from 0.3-0.8 mg l^{-1} compared with always less than 0.1mg l^{-1} at 50%. The commensurate rapid and considerable decrease in pH meant that the FAN increase was not as high as it would be at ambient seawater pH of around 8.2. Oxygen levels were not considered problematic at any stage in the experiments.

CONCLUSION

It is clear from the data that this system could feasibly run for extended periods at 75% recirculation, with relatively little effect on water quality compared with 50% recirculation. The system was run at 100% recirculation for two separate 3-day periods and, although there were no health effects observed on the abalone, ammonia reached critical levels, from literature values, on the third day. Nevertheless, the lack of obvious effects on the abalone suggests that the potential to use this seaweed bioremediation to mitigate effects of HABs could be beneficial over at least 2-3 days of a bloom event.

ESTIMATING AMMONIA-N AND CARBION DIOXIDE EMISSIONS FROM A LAND-BASED TROUT FARM: A PRECISION FISH FARMING APPROACH

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Introduction

Innovations and decrease of the cost of sensors allow the implementation of the "Precision Fish Farming" approach that can promote the ecological transition of the aquaculture sector. This approach could lead to a new generation of management software and decision support systems for fish farmers based on dynamic models.

In this work, an Individual–Based Model for Population Dynamics (IBM–PD) of rainbow trout, *Oncorhynchus mykiss*, is presented, which enables one to predict the total biomass and the fish weight distribution. The model is forced by water temperature, feed ration and feed quality. The model output includes the daily optimal feed ration, ammonia-N load and carbon dioxide emissions.

Material and Methods

The PFF approach was tested on a rainbow trout farm located in the Northern Italy. Four surveys were conducted during the H2020 project GAIN, lasting at least one month. In these campaigns, two identical probes YSI EXO2 were deployed at the inlet and at the outlet of a selected raceway for water quality monitoring, in particular: hourly measurements of Dissolved Oxygen (DO) concentration, water temperature and pH were recorded. The temporal evolution of fish weight distribution was monitored using Biomass Daily, a non–invasive device based on infrared technology that detects a signal anytime a specimen crosses a square frame. The IBM–PD was tested on mean weight and standard deviation estimated on daily observations collected by Biomass Daily. The population dynamics was simulated excluding any interaction among individuals and, hence, population weight distribution is the result of a set of individual growth trajectories. Each individual growth trajectory is simulated using a bioenergetic model (Bolzonella M. et al., 2022 – submitted). The estimation of daily loads of ammonia-N takes into account the amount of Nitrogen introduced via fish feed and the amount retained by fish to build new biomass based on body composition as suggested in literature (Dumas et al., 2010). Carbon dioxide dynamics will be simulated according to a mass balance considering contribution from water fluxes, fish respiration, exchange with the atmosphere and the chemical reactions of this chemical occurring in water.

Table 1. Comparison of NH_4^+–N emissions estimated by the IBM – PD and literature

Biomass [tons]	N content of feed	$\Delta NH_4^+ - N$	Units	Reference
10 – 16	7%	0.5 – 2.5	$kgNd^{-1}$	This work
20	6.5%	1.5	$kgNd^{-1}$	Bartoli et al., 2007
10 – 16	7%	0.02 – 0.1	$mgNL^{-1}$	This work
24	7.2%	0.045 – 0.211	$mgNL^{-1}$	Varol & Balcı, 2020

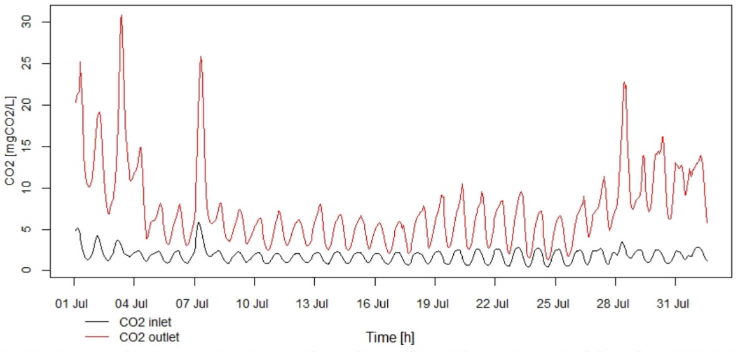

Figure 1. Estimated concentration of carbon dioxide estimated by the IBM-PD model

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Results and discussion

The ammonia-N emissions were estimated for a population with mean fish size ranging between 300g and 600g, experiencing both prolongated fasting condition and regular feeding regime. Table 1 presents the comparison of the model output with literature data. As can be seen from this table, the emissions of ammonia-N estimated by the IBM-PD are in good agreement with previous findings.

A preliminary approach based on carbonate chemical equilibria, measured pH and alkalinity was implemented in the model. Figure 1 shows the estimation of the concentration of carbon dioxide estimated by the model at the inlet (black line) and at the outlet (red line). As can be seen from this figure, the model estimated that concentration of carbon dioxide at the outlet could go outside of the optimal range for this species. The model could support trout farmers in controlling the concentration of ammonia-N and carbon dioxide in water to avoid concentrations of these chemicals above optimal level based on reared biomass and husbandry practice. The developed IBM-PD model could then be used for the estimation of the environmental burden released by a trout farm.

Acknowledgement

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MONITORING BIOMASS QUALITY OF BY-PRODUCTS FROM AN IMTA SYSTEM: EVALUATION OF HEAVY METALS

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Introduction

Marine invertebrates are known to accumulate high levels of heavy metals in their tissues (Rainbow, 2002). They can acquire metals from both water and diet, although diet has been shown to be the dominant pathway (Casado-Martinez *et al.,* 2009). The REMEDIA Life Project (REmediation of Marine Environment and Development of Innovative Aquaculture: exploitation of edible / not edible biomass, LIFE16 ENV / IT / 000343) funded by the European Community, provides, for the first time in Europe, the creation of an integrated system of multi-trophic aquaculture (IMTA) where a new set of bioremediating organisms (polychaetes, porifers, mussels and macroalgae) are placed on collectors around the fish breeding cages. In addition to the restoration activity the project foresees the biotechnological use of the bioremediator biomass, which constitute a by-product of high commercial value potentially exploitable as source of fodder, fertilizers, and bioactive compounds. Being the plant located close to the Taranto city and therefore exposed to a variety of pollutants from anthropogenic activities, the evaluation of biomass quality of some of these by-products was needed. Several important parameters, such as Pcbs, heavy metals, microplastics and bacteria concentration, have been investigated in their biomass. The present paper deals with the presence of heavy metals in the filter feeder bioremediating organisms after one year of growing in the IMTA system. The examined species were the mussel *Mytilus galloprovincialis;* the polychaete *Sabella spallanzanii* and *Branchiomma luctuosum*; and the sponge *Sarcotragus spinosulus*.

Material and methods

The study area is located on the south-west side of the Mar Grande of Taranto (40°25′56″ N; 17°14′19″ E) (Ionian Sea) (Fig. 1), which is a semi-enclosed basin connected to the Gulf of Taranto. The investigation was performed in the aquaculture plant “Maricoltura del Mar Grande (MMG)”, which is a partner of the Remedia-Life project hosting the innovative IMTA system. The investigated specimens were immediately removed from the collectors and carried in the laboratory and employed for the chemical analyses. In particular, analyses were performed on the soft tissues of mussels, the body and the branchial crown of worms and the sponge total body. Ten specimens of each species were examined. They were dehydrated for 48 h at 60 °C and their dry weights measured. Each sample was then mixed with 4 ml H_2O_2 and 6 ml HNO_3, then treated at 180 °C for 10 min using a microwave digestion system (Milestone START D). Samples were subsequently cooled, diluted with ultrapure water to a final volume of 25 ml, filtered through syringe filters (pore size 0.45 μm), and finally measured for element content by using an inductively coupled plasma atomic emission spectrometer (ICP-AES; Thermo Scientific iCap 6000 Series) Results were ultimately expressed as $\mu g\ g^{-1}$ of tissue dry weight.

Results

The obtained results are reported in Table. 1.

Discussion

All the reared organisms after one year of permanence in the plant showed lower values than those reported as not dangerous in literature except for Pb in the sponge *S. spinosulus*, and As and Cd in the crown of polychaetes. As regard sponges the high recorded concentration of cadmium does not represent a problem since their tissues were proposed only for the extraction of bioactive molecules. The high concentration of arsenic and cadmium found in the crown of both polychaete species was already reported by other authors, that suggested a biological role of toxic compounds as an adaptive mechanism against predation (Fattorini *et al.,* 2005; Notti *et al.,* 2007). In the case of polychaetes we proposed to use the entire worm when employed as ornamental animal for aquaria, by contrast we advise to remove the crown when the biomass is exploited as animal feed and as fishing baits.

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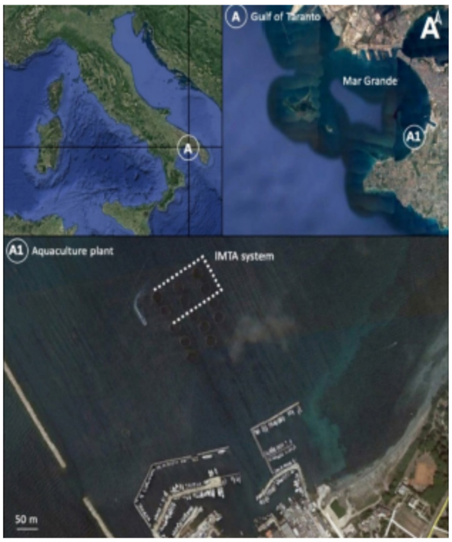

Figure 1 map of the studied site

The obtained results are reported in Table. 1.

	Heavy metal concentration (μg /g) in the bioremediators tissue					
	As	*Cd*	*Cr*	*Cu*	*Pb*	*Hg*
Reference values	*76.17*	*2.54*	*9.46*	*19.22*	*3.25*	*0.81*
Mytilus galloprovincialis	*2.29 ± 0.31*	*0.04 ± 0.01*	*0.14 ± 0.09*	*0.74 ± 0.02*	*0.16 ± 0.04*	-
Sarcotragus spinosulus	*17.88 ± 4.54*	*0.12 ± 0.02*	*2.18 ± 0.27*	*16.73 ± 1.09*	*4.03 ± 0.69*	*0.15 ± 0.18*
Sabella spallanzanii (body)	*6.89 ± 2.14*	*0.2 ± 0.04*	*0.99 ± 0.58*	*4.84 ± 0.81*	*0.45 ± 0.21*	*0.02 ± 0.02*
Sabella spallanzanii (crown)	*477.69 ± 82.73*	*2.3 ± 0.28*	*0.38 ± 0.14*	*1.53 ± 0.12*	*0.25 ± 0.08*	-
Branchiomma luctuosum (body)	*4.32 ± 0.65*	*0.12 ± 0.01*	*0.19 ± 0.22*	*3.63 ± 0.15*	*0.17 ± 0.22*	*0.15 ± 0.07*
Branchiomma luctuosum (crown)	*650.73 ± 61.36*	*2.86 ± 0.18*	*0.4 ± 0.1*	*1.73 ± 0.17*	*0.24 ± 0.04*	*0.01 ± 0.01*

Table 1. Reference values (ECR, 2006).and heavy metal concentrations in the animal tissues.

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ALTERNATIVE MATRICES FOR STRESS EVALUATION IN EUROPEAN SEABASS (*Dicentrarchus labrax*) EXPOSED TO HYPOXIA

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Introduction

In aquaculture, fish undergo a series of events and practices that can compromise their welfare and health. One of the most frequently used stress indicators in fish is the quantification of cortisol in conventional matrices, such as plasma. Nevertheless, blood sampling can be extremely invasive, becoming itself a stressor and cortisol quick increase after stress may lead to unreliable results. This is why non-conventional matrices such as muscle, fin and skin mucus have already been tested as valuable alternatives to plasma (Bertotto et al., 2010; Sadoul & Geffroy, 2019). Among them, scales have also been recently taken into consideration as less invasive matrices and as novel and reliable marker for chronic stress in European sea bass and other species (Samaras et al., 2021). Cortisol in scales is accumulated slowly, due to their bony nature, making them unsuitable for acute stress studies but essential to evaluate fish stress response over the mid-long term. This study aims to evaluate the stress status of European seabass subjected to hypoxia by quantifying cortisol levels in a suite of matrices using specific radioimmunoassays (RIAs). For a deeper insight into the stress response, measurements of hematocrit and oxidative stress were also taken.

Materials and methods

European sea bass (*n*=60; 158±10 g) were acclimatized for 4 weeks at 22.5±0.5°C and 8.5±0.5 mg/L of oxygen. Fish were then randomly distributed in 6X1000L tanks with a daily renewal rate of approximately 5%. The experiment was performed at the Department of Veterinary Medical Sciences (University of Bologna, Italy) and during the whole period temperature was kept at 26±1°C and the photoperiod was set at 12L:12D. Three tanks were used as control and had normal oxygen levels (8.7±0.4 mg/L), whereas the other three had low oxygen levels (4±0.2 mg/L) to establish hypoxic stress. Fish were sampled at the beginning of the trial, before exposure to hypoxia, and at the end of the trial, which lasted 7 days. During the samplings, fish were sacrificed by excess of anesthetic (MS222 Sigma-Aldrich) and subsequent cut of the spinal cord. Blood was collected from the caudal vein using heparinized tubes and samples of skin mucus and scales were collected by scraping the side of the fish. Immediately after collection, two aliquots from each blood sample were taken for hematocrit measurements, while the remaining part was centrifuged to obtain plasma samples. Small portions of lateral muscle ($1cm^3$) and caudal fin ($1cm^2$) were also collected. All the matrices were subsequently stored at -20°C until the analysis.

Cortisol was measured in all the matrices by a previously validated specific RIA protocol for use in European seas bass (Bertotto et al., 2010). For the scales, different extraction methods were tested and a specific RIA protocol was developed and validated. Plasma was also used for the assessment of lipid peroxidation, i.e. malondialdehyde (MDA), by measuring thiobarbituric acid-reactive substances (TBARS). All the data are expressed as mean ± standard error and were previously evaluated for normality distribution. Differences between treatments were analyzed using a general linear model (GLM) using *time* and *hypoxia stress* as main factors. Correlations in cortisol concentrations between plasma and the various matrices were performed using Pearson's correlation coefficient. The level of statistical significance was set at $p < 0.05$.

Results and discussion

The RIA protocol developed to assess cortisol in the scales showed good parallelism and reproducibility (linear regression curve coefficient R^2: 0.99; CV% intra-assay = 5.14; CV% inter-assay = 4.39) and the recovery test with value higher than 90% confirmed the efficiency of the method for steroid extraction.

Results showed that cortisol concentration in plasma, fin and skin mucus increased over time, although not significantly (p=0.21, p=0.27 and p=0.07, respectively). Neither the exposure to hypoxia affected the hormone levels in the same matrices (p=0.92, p=0.90 and p=0.18, respectively). On the other hand, a significant increase in cortisol concentration in muscle and scales was observed in fish over time (p<0.01 and p=0.001, respectively), but no differences were found in fish exposed to hypoxia stress compared to controls (p=0.83 and p=0.88). As scales are composed of bone tissue and slowly accumulate cortisol, they are little influenced by the acute effect of the sampling and allow to quantify the level of stress in the mid-long term. However, the low oxygen conditions used in this trial were too mild to trigger a strong stress response

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and a high increase of blood cortisol, whereas the scales probably seized the low increase of cortisol during the prolonged mild stress response or the increase of cortisol during the first sampling. For the same reason, hematocrit values did not differ (p=0.1) in relation to oxygen conditions, whereas the hematocrit decrease (p<0.05) over time could be a sign of anemia. The correlation of cortisol levels in plasma and in the alternative matrices, i.e. muscle, fin and mucus, was evident and significant (p<0.001, r=0.68; p<0.001, r=0.72 and p<0.001, r=0.60, respectively). Correlation between plasma and scales cortisol levels was also positive, even if less marked due to the different meaning of the hormone in these matrices in term of acute and long stress (p<0.05, r=0.36). Finally, plasma MDA concentrations were not significantly affected neither by the time nor by hypoxia and were similar to those already reported for European sea bass (Vinagre et al., 2012), highlighting the absence of oxidative stress.

Conclusions

The present study confirmed that scales could represent one of the most effective matrices to quantify cortisol levels and to evaluate the effects of sub-chronic and chronic stress, even in mild conditions. The hypoxia conditions applied in this study did not strongly impact European sea bass stress response, indicating that this species might have a high tolerance to poor water oxygenation. Nevertheless, these results need to be confirmed in future trials evaluating a further decrease of oxygen concentration and longer-term effects (beyond 7 days) both in terms of stress response and overall performance of fish.

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MULTILOCUS ANALYSIS OF INTRON POLYMORPHISMS AS A PROMISING AND ECLECTIC TOOL FOR GENETIC CHARACTERISATIONS

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Introduction

The interest for the use of diagnostic molecular markers to genetically identify species and interspecific hybrids is increasing with both commercial and conservation purposes. Lowering costs and processing time is of primary relevance especially for routine applications in which pureness of individuals must be rapidly assessed such as in the enforcement of commercial controls and in the selection of captive breeders (Toews & Brelsford, 2012; Camacho-Sanchez et al., 2020).

We present a new class of nuclear molecular markers carrying interesting features potentially useful to investigate different levels of fish genetic diversity: introns' polymorphisms.

Material and Methods

Based on an *in silico* analysis of available fish genomes, we isolated several intronic regions potentially conserved across teleosts to be characterized by an opportunely optimized targeted-amplicon high-throughput sequencing method.

A total of 122 intronic loci were selected for primer construction. All primer pairs were validated on 66 species also including important species for aquaculture and conservation purposes. Sequencing data allowed for evaluating suitability and reliability of such introns in detecting species and differentiating populations. We will also present a case study on the discrimination between the *Solea solea* and the very similar *S. aegyptiaca*.

Results

Introns has proven to be highly transferable across species with about 70 loci successfully amplified in over half of the species tested and their sequence variability has proven to have a very high-resolution power in differentiating at the species level. Focusing on the case study of the genus *Solea*, we identified 38 perfectly diagnostic loci with alternative private alleles between the two species. that These loci allowed for setting up fast and cheap identification tool base on introns' polymorphisms.

Discussion

We presented the first panel of highly conserved intron markers, characterized by high transferability between species, and variability in terms of allelic richness, as well as remarkable diagnostic potential for analysis of species and hybrid identification. Obtained results are very encouraging also for many other applications such as detection of population origin and relatedness analysis. What showed in the case study can be optimized for many other fish species.

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CAN FOULING SEAWEEDS ON SEA-BASED STRUCTURES ADD VALUE TO AQUACULTURE? A BIODIVERSITY SURVEY AND SEA-URCHIN FEEDING STUDY

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With the increase of aquaculture production, sea-based farming and culture practices must innovate to meet economic and environmental challenges for tomorrow. With more than 10 000 ha of maritime concessions along the French coasts of North Atlantic and Western English Channel, developing aquaculture sectors from a sustainable perspective, by promoting polyculture and IMTA, has become a priority for local authorities. This on-going project aims to guide aquaculture practices to further integrate sea-based structures into marine ecosystems, by using them as support for wild species.

Sea-based aquaculture structures are usually colonized by an important diversity of fouling species including seaweeds. Considered as undesirable, fouling seaweeds are the results of local recruitment and can include species displaying an economical interest for different production sectors (*e.g*. food industry, material, aquaculture). In this study, we explore the potential of ecosystemic services of fouling seaweeds to highlight the contribution of sea-based structures i) for the conservation and dispersal of local biodiversity and ii) for providing add-value seaweed species for farmers. We particularly explore the integration of fouling seaweeds in technical itineraries of sea-based breeding and cultivation, by:

- Evaluating the succession of fouling seaweeds on blank longlines
- Assessing the development of fouling seaweeds on sea-based seaweed culture by comparing blank longlines with seeded longlines of *Saccharina latissima* and *Palmaria palmata*
- Comparing the effect of feeding sources, *i.e*. fouling *versus* cultivated seaweeds, on a sea-based sea-urchins rearing

For this purpose, 200 m of seeded (100 m per species) and 200 m of blank longlines were installed on a sea-based seaweed cultivation structure in January 2022. Eight cages of 0.3 m^3, each containing 25 sea-urchins, were added to the structure in April 2022. Sea-urchins are fed every 15 days, four cages with cultivated seaweeds and four cages with fouling seaweeds. Specific Richness and Relative Biomass of seaweeds on both longline types, as well as Mortality Rate, Relative and Specific Growth Rates, Food Consumption and Conversion Ratios in each sea-urchin cages, are evaluated every month.

After 2 months, a total of 12 taxa were identified on longlines: 8.0 ± 1.0 on fouling longlines, 2.3 ± 0.6 and 5.0 ± 1.7 on *S. latissima* and *P. palmaria* cultivated longlines, respectively. As expected, seeded longlines are dominated by the cultivated species reaching biomass values (wet weight) of 460.2 ± 32.2 $g.m^{-1}$ and 296.6 ± 162.3 $g.m^{-1}$ for *S. latissima* and *P. palmaria*, respectively (Figure 1), while fouling longlines are dominated by brown algae*, i.e*. diatom colonies and filamentous species (*Hincksia sp*., *Pylaiella sp*.).

Next results of seaweed biodiversity and first data on the sea-urchin rearing will be obtained during May 2022, and will provide first insight of the role of fouling seaweeds in a sustainable approach of off-shore aquaculture.

Figure 1. Pictures of Saccharina latissima and Palmaria palmata cultivated longlines

BILE IMPRINT ON THE PARIETAL PERITONEUM IN RED SEABREAM AND GILTHEAD SEABREAM: EFFECTS OF DAYS OF FASTING, STRESS AND ICE-STORAGE

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Introduction

Recently, a common complaint of Mediterranean farmed fish retailers is the occurrence of the bile imprint on the parietal peritoneum observed when fish are eviscerated or filleted. This bile mark is unattractive to the consumer who may also complain about a bitter taste of the fish flesh. The bile imprint has occurred in a more frequent and intense way in farmed red seabream, although present in the other Mediterranean farmed fish also. To our knowledge there are not related published works and the cause has not yet been identified. The aim of the present study was to investigate the possible involvement of common procedures applied before fish harvesting (i.e., days of fasting, stress due to confinement and netting), as well as ice-storage of fish, on the occurrence of the bile imprint in red seabream *Pagrus major* and gilthead seabream *Sparus aurata*. Besides, plasma and bile osmolality and cholesterol levels were evaluated in search of a reason of bile diffusion to the peritoneum.

Materials and methods

Two factorial experiments were designed, one for red seabream and one for gilthead seabream. For each trial 144 fish of mean initial body weight 185±1.3 g (red seabream Trial) and 200±2.8 g (gilthead seabream Trial) were randomly distributed, in groups of 6 fish, in 24 tanks. Fish were fed at a fixed ratio for approximately 3 weeks. At the end of the rearing, 8 tanks were randomly assigned to 1 day of fasting (DoF), another 8 tanks were assigned to 2 DoF and the last 8 tanks were assigned to 3 DoF. For each DoF, 4 tanks were sampled undisturbed and 4 tanks were stressed by confinement for 20 minutes. All fish were euthanized by an overdose of anaesthetic and were individually weighed and measured. Blood (centrifuged to separate plasma) and bile sampling took place from the first three fish of each fish group (0 h of ice-storage). The other 3 fish of each fish group, whole and ungutted, were stored in refrigeration at 2±1 ^{o}C for 48 h (48 h of ice-storage). They were placed in polystyrene boxes provided with outlets for water drainage, covered with protect-ice plastic film and filled with ice. Fish packaging was done according to the protocol followed in commercial production, i.e. 20 fish were packed in one 50 x 30 x 16 cm box. After 48 h of ice-storage, bile sampling took place. For both 0 h and 48 h fish, parietal peritoneum was photographed in LED Photo Box to ensure similar lighting conditions. Plasma and bile were analyzed for osmolality and cholesterol. Colour evaluation of the bile imprint, if present, was performed by measuring optical density of the imprint by means of digital image analysis software.

Results

Red Seabream Trial. None of the 0 h fish had a bile imprint. Instead, after 48 h of ice-storage a bile imprint was present in all the fish. Colour of the bile imprint was greatly affected by number of days of fasting, i.e. the more the DoF the more greenish the bile imprint colour. Moreover, the imprint was more intense in stressed fish no matter days of fasting. Bile osmolality was not affected by DoF. In 0 h fish it was significantly lower than plasma osmolality and was not affected by stress, while plasma osmolality was higher in stressed fish. After 48 h of ice-storage bile osmolality was highly increased, especially in stressed fish. Regarding cholesterol (0 h), confinement stress resulted in higher levels in plasma and did not affect bile levels. Days of fasting affected bile cholesterol and 3 DoF resulted in lower levels compared to 1 DoF. Generally, bile cholesterol (0 h) was lower than plasma cholesterol. In 48 h fish, bile cholesterol was significantly reduced, especially in unstressed fish.

Trial GS. Currently, the Trial has just been completed and samples and photos are being processed.

Discussion

The present “incomplete” abstract will be replaced in due course.

BLUE FOOD PLANNING AND CLIMATE CHANGE: FOOD WEB MODELS AS TOOLS FOR THE ANALYSIS

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Introduction

Forecasted long-term trends in environmental parameters, induced by climate changes, need to be considered in the ongoing and future management of blue food productions (*sensu* Blue food assessment, 2022), in order to attempt a sound adaptation to changes that are likely to affect these activities (Cochrane et al., 2009; FAO, 2015). Here we apply a dynamic food web model in a marine coastal area located in the Gulf of Venice (Northern Adriatic Sea), were longline mussel farms (Mediterranean mussel, *Mytilius galloprovincialis*) coexist with different types of fisheries, including, recreational, artisanal, clam dredging and trawling, and with important conservation assets, including a marine rocky bottom Natura 2000 site. Our final aim is to produce forecasts considering both scenarios of climate change and interaction of aquaculture with other uses of the sea, in order to support the analysis of future blue food production potential in the area in the framework of maritime spatial planning.

Methods

An existing food web model (based on Ecopath with Ecosim) of the northern Adriatic Sea was adapted to this coastal area (Donati et al., 2021), characterised by the presence of mussel farms, a sandy bottom hosting clam beds of high commercial importance, and biogenic rocky outcrops relevant for biodiversity conservation. 28 trophic groups were defined in the model, see Figure 1, considering different habitats and tolerance to environmental drivers. Mussel farming was characterised based on previous studies in the area (Brigolin et al., 2009). Fishing activities was described considering 6 different fleets (including different trawling gears, hydraulic dredge, artisanal and recreational fishery). Dynamic food web simulations were performed by introducing time series of water temperature and primary production (climate change) and fishing effort/aquaculture production (bluefood management). Simulations considered a time frame of 40 years, from 2014 to 2054, comparing 2 scenarios RCP, 4.5 and 8.5. Downscaling for forcing variables were taken both from the EURO-CORDEX CMIP5 and from biogeochemical model outputs (ERSEM), produced in the framework of the CERES H2020 project. The FWM provided as an output time series of group biomasses, catches, and ecosystem functioning indicators. The temporal variation of ecosystem functioning indicators and of trophic groups biomasses was assessed with respect to a baseline scenario.

Results and discussion

Tolerance curves identified (Figure 2left) and used in the dynamic FWM allowed to derive a comprehensive set of ecosystem functioning indicators under the two CC scenarios considered (as shown in Figure 2 right, considering only temperature induced changes). Higher percentage of variation was presented by those indicators associated to groups biomasses, as the commercial B and Kempton (K), showing decrease ranging between 10 and 15%. Indeed, different groups including species of high commercial interest presented a substantial biomass decrease. Results provided differing assessments when considering only variations in mussel aquaculture and fishery productivity, or the overall ecosystem functioning of the area. This is discussed with regard to the ecological carrying capacity concept.

Acknowledgements

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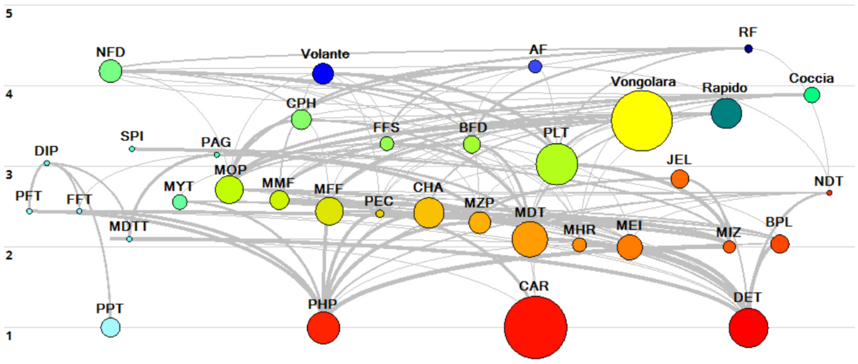
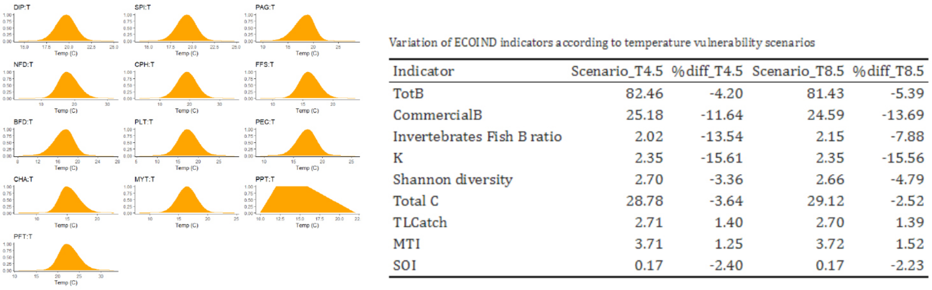


*Figure 1. Food web structure. Circle width and lines thickness is directly proportional to the trophic group abundance (t*km-2)) and to the amount of organic matter exchanged within the trophic interaction, respectively. Numbers on the left axis indicate the TLs in which the different groups are positioned.*



Variation of ECOIND indicators according to temperature vulnerability scenarios

Indicator	Scenario_T4.5	%diff_T4.5	Scenario_T8.5	%diff_T8.5
TotB	82.46	-4.20	81.43	-5.39
CommercialB	25.18	-11.64	24.59	-13.69
Invertebrates Fish B ratio	2.02	-13.54	2.15	-7.88
K	2.35	-15.61	2.35	-15.56
Shannon diversity	2.70	-3.36	2.66	-4.79
Total C	28.78	-3.64	29.12	-2.52
TLCatch	2.71	1.40	2.70	1.39
MTI	3.71	1.25	3.72	1.52
SOI	0.17	-2.40	0.17	-2.23

Figure 2. left) tolerance curves used to simulate the response of the system to CC; right) values of indicators obtained by ECOIND, for Ecosim, at the end of the simulations, for the T4.5 and T8.5 scenarios, including variations relative to the baseline scenario considered.

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POTENTIAL RECYCLING OF PHOSPHORUS FROM SALMON FARMS WITH *Saccharina latissima* CULTIVATED IN IMTA

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Introduction

Future scarcity in phosphorus resources is a challenge directly linked to food security, and solutions are needed to shift the current phosphorus use into more sustainable forms (Cordell et al., 2009, 2011). The release of biogenic waste from finfish aquaculture along coast lines can have negative impacts on marine ecosystems (Jansen et al., 2018), as well as being a wasteful practice with limited resources like phosphorus. Recirculation of biogenic waste from aquaculture can be done by co-cultivating species like *Saccharina latissima,* in integrated multi-trophic aquaculture (IMTA) systems (Chopin et al., 2001). This study characterizes storage of phosphorus in *S. latissima* cultivated close to salmon sea cages and evaluates the recycling potential of this species.

Materials and methods

A 4-month cultivation study was conducted by deploying vertical ropes (5 m) with *S. latissima* seedlings along a gradient within and downstream from a salmon farm. Ropes were grouped as integrated among pens (I-SL, -150 to 0 m), and downstream from farm edge (D-SL, 100 to 280 m), assuming different nutrient availability between groups from farm proximity. Biomass registration, water- and tissue samples were collected and analyzed after 2 (April) and 4 (June) months in the sea. Tissue samples were analyzed for carbon (C) and nitrogen (N), and phosphorus (P) content from three areas of the seaweed to characterize internal distribution: basal- (B), mid- (M), and apex area (A).

Results and discussion

Tissue content of P (Figure 1) varied significantly between I-SL and D-SL group. The content was highest in the B area in April for both groups ($p<0.05$), indicating prioritized accumulation of nutrients in the meristematic tissue to facilitate growth in young individuals. This support phosphorus as an important element for physiological functions involved in growth (Hurd et al., 2014). The A and M area was significantly different ($p<0.05$) in the D-SL group but not for the I-SL group, indicating an effect of nutrient availability affecting internal content of P. Overall, the magnitude of P content suggest that algae cultivation solely for recycling P is not recommended.

Biomass yields were significantly higher in the I-SL with a peak yield of $7.01\pm0.88\ \mathrm{kg_{FW}m^{-1}}$ in June, supporting studies with increased biomass yield by cultivation near salmon farms (Marinho et al., 2015). However, within the I-SL group biomass yield was highest at the farm edge, decreasing further in-between salmon pens (Figure 2). Suggesting that other factors could be limiting light and water movement patterns, affecting seaweed growth (Klebert et al., 2013). This supports cultivation of seaweed closely downstream from salmon farms, ensuring high biomass yield. Biomass yield was promoted the main factor for estimating bioremediation potential.

Conclusion

Understanding of biochemical composition, and how it relates to growth is important when establishing the seaweed cultivation industry. Nutrient availability can be an important factor, but this study also shows how physiological mechanisms regulate internal nutrient availability to ensure rapid growth at the right time.

The cultivation of *S. latissima* in IMTA systems can be an opportunity for recapturing phosphorus in a production of biomass for a new value chain. However, phosphorus content by itself is not an ideal measure for assessment of bioremediation potential.

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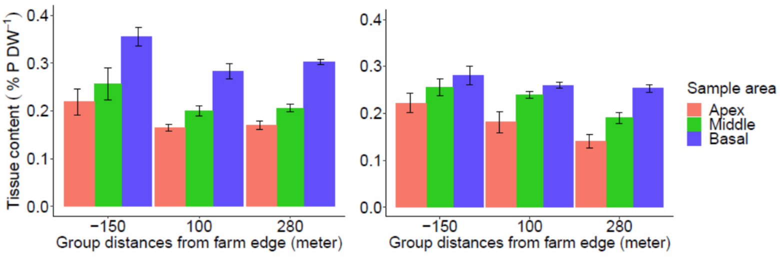

(a) April

(b) June

Figure 1: Variation and distribution in phosphorus tissue content in *S. latissima*, given as mean+/-CI95% for different sample areas of the lamina and at different distances from the fish farm edge.

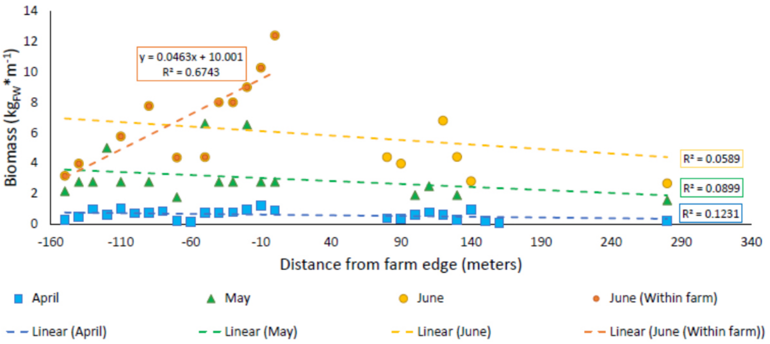

Figure 2: Rope biomass yield ($kg_{FW}m^{-1}$) at different distances from the farm edge for three sampling days. Slopes for estimation of change in biomass yield across the gradient, with R^2 are included. The I-SL group in June is highlighted, with a fitted slope and R^2.

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USING SEA URCHIN FAECES AS A DIETARY SUPPLEMENT FOR JUVENILE ABALONE: EFFECTS ON GROWTH, SURVIVAL AND THE MICROBIOME

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Introduction

During the early post-settlement juvenile stages, the abalone *Haliotis midae* has a known ecological relationship with the Cape sea urchin *Parechinus angulosus* in nature. The urchins provide the juvenile abalone with shelter and protection, as well as with improved food supply and possibly provide beneficial microbial communities, all of which could act as drivers of the relationship between these species. These species are therefore strong candidate species for integrated multi-trophic aquaculture (IMTA), as they also have a similar preferred temperature range (12 - 20°C). Furthermore, hatchery-produced abalone could benefit from sea urchin faecal matter supplementation during early juvenile stages as the transfer of microbial communities could aid in digestion, growth and overall health. Therefore, the aim of this study was to evaluate the potential benefits for abalone growth, survival and subsequent changes in the microbiome associated with using sea urchin faecal matter as a supplementary feed or probiotic for juvenile abalone.

Materials and Methods

An 8 week experiment was conducted in a commercial hatchery in South Africa (Buffeljags Abalone) where two sets of three tanks, each tank (L x W x D: 0.68 m x 0.50 m x 0.12 m) with 25 000 juvenile abalone with starting shell lengths of ca. 3 mm that were fed a mixed diet of diatoms, formulated feed, *Ulva lacinulata* and *Gracilaria*. One set of tanks was only fed the mixed diet (control) and the other set was provided with a daily supplement of sea urchin faecal matter collected from 310 sea urchins per tank. Sea urchins were only fed *U. lacinulata* and were held in baskets in separate tanks (L x W x D: 0.68 m x 0.50 m x 0.12 m) as preliminary trials indicated that this simplifies animal husbandry practices for these species.

Juvenile abalone growth and survival was monitored for the duration of the trial. Abalone growth was assessed by measuring shell length and width (in cm) of 300 randomly chosen animals per tank at each timepoint (0 weeks, 1 week, 4 weeks and 8 weeks). Survival was assessed weekly by collecting and counting mortalities per tank. Using the Illumina MiSeq next-generation sequencing platform, a 16S rDNA and ITS2 metagenomics approach was used to characterise bacteria, fungi and oomycete communities. Metagenomic samples (n = 75) included incoming seawater (500 mL) that was collected on 0.22µm filters, sea urchin intestines and faecal matter, abalone intestines and faecal matter, as well as each of the feeds. Once raw sequence data is received, data will be processed using QIIME2 (Boylen et al., 2019). Bacterial 16S reads will be mapped against the SILVA 16S rRNA reference database (Quast et al., 2013) for taxonomic identification. Fungal and oomycete ITS2 reads will be mapped against a custom fungal and oomycete database that included the UNITE database (Abrenkov et al., 2021) and oomycete sequences downloaded from the National Center of Biotechnology Information (NCBI). MicrobiomeAnalyst will be used to assess within- and between sample diversity, as well as to quantify and visualise taxonomic abundance and differential abundance analyses (DESeq2). Lastly, the functional capabilities of the identified taxa will be assessed.

Results & Discussion

Results from the large-scale trial showed that the shell length of juvenile abalone were statistically significantly larger ($P < 0.05$; one-way ANOVA) at 1 week, 4 weeks and 8 weeks (Figure 1), with shell width being statistically significantly larger at 1 week and 4 weeks.

Although there were limited statistically significant differences in survival, the growth results suggest that the faecal matter and possibly the microbes transferred from the urchins to the abalone provides an advantage in the early post-weaning stages. This trophic transfer of microbial communities will be further assessed in this study. Enteric bacteria are thought to play roles in the synthesis of essential amino acids, provisioning of digestive enzymes, improvement of digestive efficiency, nitrogen metabolism, as well as access to essential micronutrients.

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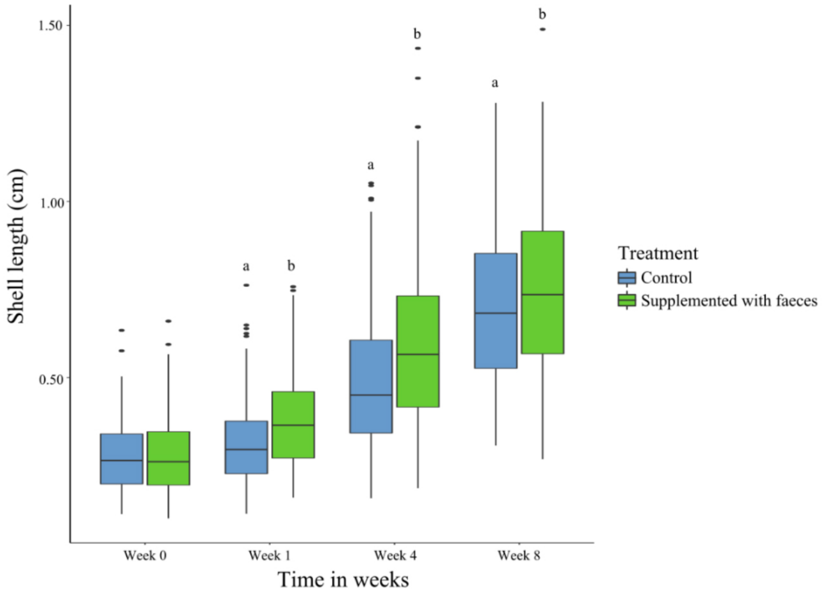

Figure 1. Average juvenile abalone shell length (cm) over a duration of 8 weeks across abalone fed only diatoms, a formulated feed, *Ulva* and *Gracilaria* (control, n = 300 per time point) and abalone fed diatoms, a formulated feed, *Ulva* and *Gracilaria* while being supplemented with sea urchin faeces (n = 300 per time point).

Conclusion

Supplementing juvenile abalone diets with sea urchin faecal matter is advantageous for abalone growth and health in their early juvenile stages, possibly as a result of microbial community transfer from the sea urchins. The intestinal microbiome is often established during the juvenile stages for aquatic animals and could have long-lasting effects on digestion, overall performance and health, possibly through the microbial provisioning of digestive enzymes. This study will provide insight on the potential benefits and effects of supplementing juvenile abalone diets with sea urchin faeces.

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AN ENVIRONMENTAL ASSESSMENT OF AN AQUACULTURE SYSTEM IN PORTUGAL FROM LIFE CYCLE PERSPECTIVE

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Introduction

Within the food sector, aquaculture has its share in supplying animal-source food and protein worldwide. Fish harvested from aquaculture amounted to 73.8 million tonnes, accounting for 44% of total aquaculture production, contributing this way to meet current needs (FAO, 2016). The overall aquaculture production in Portugal was 16,999 tonnes in 2020 with the vast majority taking place in marine and transitional waters (coastal ecosystems) (INE, 2021).

To date, aquaculture systems have been poorly assessed in Portugal from an environmental perspective, and in particular, using Life Cycle Assessment (LCA). LCA is a widely applied methodology that determines the potential environmental burden of products and services throughout their entire life cycle. Additionally, the linkage of resources, food and nutrition are likewise poorly addressed in the literature. This study aims to analyse the environmental impacts of a small-scale aquaculture system in Portugal by applying LCA.

Materials and Methods

The aquaculture system (AQS) is located in the Ria de Aveiro which is classified as a coastal lagoon ecosystem. The AQS is a semi-closed-extensive system and comprises 3 natural ponds, with a net area of 28 hectares (ha). It produces seabass (70%) and seabream (30%), being the production cycle of 18 months.

The LCA methodology follows the standards ISO 14040 and ISO 14044 (ISO, 2006)"title":"Environmental management. Life cycle assessment. Requirements and guidelines.","type":"report"},"uris":["http://www.mendeley.com/documents/?uuid=b1102395-0dee-41cb-8faf-6ec7c94fc4e4"]}],"mendeley":{"formattedCitation":"(ISO, 2006. Based on the product output, 1 kg of medium size fish (MSF) was chosen as functional unit (FU). The system boundaries encompassed the pre-production that includes the transportation of juveniles and the production that consists on fish fattening and growth. Primary data were directly collected at the aquaculture farm by means of questionnaire and by interview. As environmental impact assessment method, it was adopted the Environmental Footprint method that is proposed by the European Commission (Fazio et al., 2018). The impact categories included were climate change (kg $CO_{2\text{-eq}}$), acidification (mol $H+_{eq}$), freshwater eutrophication (kg P_{eq}), marine eutrophication (kg N_{eq}), water use (m^3 deprivation) and fossil resource use (MJ).

Results

The overall results indicated that the majority of the impacts are concentrated in the production process (Table 1). Moreover, within this process, the production of fish feed represented the largest share (99%) of the total impact in all impact categories. The life cycle impacts are presented in the table below. In terms of resources, the results showed a very high dependency on abiotic n on-renewable resources (fossil fuels), accounting for 59% of the total resource impact. Fish soy-based feed production has the largest share in the consumption of fossil fuels.

Table 1 – Impact assessment results expressed per FU

Impact category	Unit/FU	*Pre-production*	*Production*	Total
Climate change	kg CO_{2eq}	9.28E-03	2.87E+00	2.88E+00
Acidification	mol $H+_{eq}$	2.30E-05	2.71E-02	2.72E-02
Eutrophication, freshwater	kg P_{eq}	6.65E-07	5.56E-04	5.56E-04
Eutrophication, marine	kg N_{eq}	4.47E-06	8.93E-03	8.94E-03
Water use	m3 depriv.	1.18E-03	3.99E+00	3.99E+00
Resource use, fossils	MJ	1.65E-01	2.38E+01	2.40E+01

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Conclusions

Although the results are in the same order of magnitude concerning LCA studies available in the literature, the assessment of the culture of seabass and seabream revealed lower impacts than the majority of existing case studies. Notwithstanding, the impacts cannot be overlooked, mainly from the fish feed. Therefore, it is suggested to look for strategies that ensure the long-term environmental sustainability of this type of aquaculture system. These strategies could point towards incentivizing small-scale and local organic aquaculture, using more sustainable fish feed, and seeking to benefit from the natural conditions by harnessing provisioning and regulating ecosystem services, thus increasing the renewability of the systems.

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GIS APPLICATION FOR OFFSHORE AQUACULTURE SITE SELECTION ALONG THE RED SEA, SAUDI ARABIA

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Introduction

Early aquaculture practices have failed thus far to incorporate environmental impacts and sustainability into their establishment and production plans, resulting in numerous negative environmental (Fernandes, Eleftheriou et al. 2001) and long-term production impacts, inefficient production cost effectiveness and negative public views about aquaculture practices (Froehlich, Gentry et al. 2017). However, industry, regulatory agencies, and public mindsets have recently shifted towards more sustainable aquaculture practices and technologies that primarily include the identification of suitable sites to be matched with the assessment of their carrying capacity. A geographic information system (GIS) creates, manages, analyses, and maps all types of spatial data. This provides a foundation for mapping and analysis that is used in science and almost every industry. This study uses GIS to identify sustainable sites for aquaculture development: using numerous criteria and parameters. Several sites suitable for offshore aquaculture development were identified along the Saudi territorial waters.

Material and methods

On the basis of pre-existing usage, a relevant part of the Saudi territorial waters was excluded from the number of available areas for offshore aquaculture.

The site selection process started by establishing a panel of scientist and aquaculturist experts in the domain in Saudi Arabia, with the aim of defining the most relevant criteria to be used in aquaculture site selection. Due to the Red Sea specificity, the agreed criteria were:

1. Bathymetry
2. Port distance
3. Reef distance
4. Current average
5. Naval traffic
6. Coast distance

For each of these criteria, a range of values were established, classifying aquaculture sites as:

- Optimum, score 1
- Average, score 2
- Poor, score 3

Those scores were subsequently weighted, assigning them an average weight on the basis of the expert's judgement of their importance in running an aquaculture activity in Red Sea. The assigned weight ranged between 1 (maximum weight) to 0 (minimum weight).

The areas identified as suitable for aquaculture activities were then partitioned by a 500x500 m grid. Each of the squares were finally ranked, assigning each one a final score for each criterion, represented by the initial score multiplied by the weight assigned by the expert panel.

The sum of the final scores, over the whole available criteria, determined the final tier to be assigned to each 500x500 m square over the map. This sum ranges from 4.3 to 12.6.
The final tiers were assigned as follow:

- Up to 4.3, optimum overall conditions, tier 1, color green;
- Up to 8.6, average overall conditions, tier 2, color yellow;
- Up to 12.9, poor overall conditions, tier 3, color brown.

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Results

The GIS exercise covered most of the Red Sea and allowed for the identification of several areas suitable for offshore aquaculture. These areas were further classified on an optimum- average-poor scale on a 500x500 m grid and displayed on the map in three different colors (Figure 1).

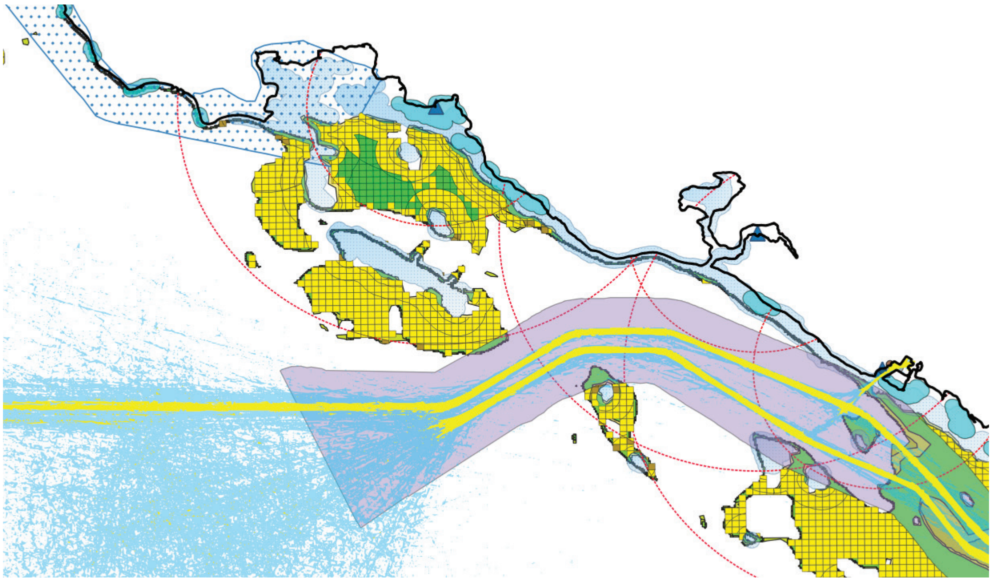

Fig. 1 *GIS output for a section of the Saudi Arabian Red Sea Coastline*.

Most of the areas suitable for offshore aquaculture are located in the southern region of Saudi Arabia, characterized by extensive shallow bottom, gentle sloping topography and surrounded by coral formations.

Conclusion

The uses of the Red Sea in Saudi Arabia are mainly driven by decision taken at the of central or local authority level. This shortens the process of assessing the aquaculture compatibility of areas and makes the selection process rather different from those carried out elsewhere (Marino et al., 2020). This exercise in fact mainly dealt with the areas left open by other existing activities, and most of the work focused on identifying the marine activities and the sensitive areas to be buffered, also assessing the reasonable buffer extent. The coral reefs were identified as the most sensitive communities to be protected due to their endangered status at the global level and to their specific sensitivity to nutrients. The value of corals reefs for Saudi economy is particularly huge, and for these reasons a double buffer level, the first derived from literature, the second, more conservative, from field observation, was set.

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RED SEA FISH FARMING ECOSYSTEM EVALUATION: A FISH FARM ANALYSIS SYSTEM TO MANAGE SUSTAINABLE DEVELOPMENT OF THE AQUACULTURE INDUSTRY ALONG THE RED SEA WATERS OF SAUDI ARABIA

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Introduction

Upon request from Saudi MEWA, King Abdullah University of Science and Technology is initiating comprehensive and detailed work to design and engineer a sustainable offshore fish farming in the Red Sea. The dedicated team will evaluate the environmental carrying capacity and optimal farming operations at six selected sites along the Saudi western coast. Farmed species at these sites will be selected from 5 species candidates. Field surveys will be conducted and fish farm models will be run to simulate operations and environmental impact for each of the sites. The simulations will be run using AquaModel, an advanced 3-dimensional, ecological and operational model that has been developed over the last 20 years by System Science Applications (see AquaModel.net). Stakeholders around the world have used this software, which runs on Windows PCs, for environmental monitoring, siting, licensing, and addressing regulations.

AquaModel provides a 3-dimensional simulation of growth and metabolic activity of multiple farmed finfish species along with associated flow and transformation of nutrients, oxygen and particulate wastes in waters surrounding the farm. The software is the first and only computer model for net-pen aquaculture and kelp growth in the world that can simultaneously calculate and display real-time feed ingestion, growth, respiration and egestion by cultured organisms. AquaModel has simulated fish farm operations for 11 species of finfish as well as sugar kelp. Fig. 1 shows processes that are described in the 3-dimensional simulation.

Materials and Methods

Data from the field surveys, output from KAUST's Red Sea circulation model, and laboratory measurements of species growth and metabolism will be ingested into site specific project files. Inputs to the simulations will include a high-resolution 3-D ocean current data, bathymetry and coastline, presence-absence of coral reefs and nearby beaches, and ambient sediment conditions such as sea bottom grain size, bottom water oxygen and nitrogen, hydrogen sulfide, as well as TOC and POC. Oceanographic inputs to the simulation will also include as best as possible time series of water temperature, chlorophyll, mixed layer depth, oxygen, nitrogen, salinity, irradiance, and winds. The simulations will also include operational variables of the farms within the 6 sites. These include location, number of pens, spatial coordinates of each pen, height/length/depth dimensions of each pen, cultured species, duration of operation. Stocking, feeding and harvesting schedules for each farm will also be entered into the calculations. Physiological models for four finfish species will been created using available literature as well as farm and laboratory data. A plankton sub-model that runs in AquaModel will be expanded and parametrized to include model parameters for both large and small ensembles of phytoplankton and zooplankton characteristic of the Red Sea.

Results

Figure 2 shows screenshots of model output concerning one site 5 km offshore along the southern coast. The left panel of figure 2 is a screenshot of the flow field at a single step of the simulation. The small cross seen offshore is the location of the cages of the farm. The plot on the left of the screen or a control panel for viewing the simulations and the panel below is used to set-up operations of the farm. The scale of current velocity in the flow field are shown on the right. The right panel is a zoomed in view of farm and shows cages, a dissolve nitrogen plume from the cages, current vectors. On the left are plots of the depth distribution of the nitrogen plume, a time series of current velocity and the increase in fish biomass of the farm. On the left are legends for current velocity, nitrogen concentrations at the sea surface, and detailed information on conditions within selected cages.

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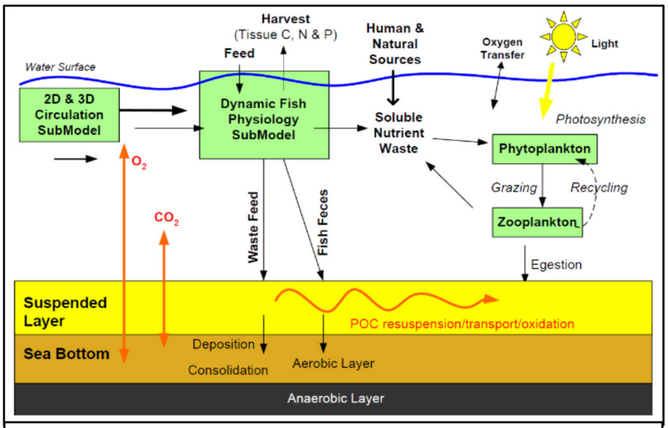
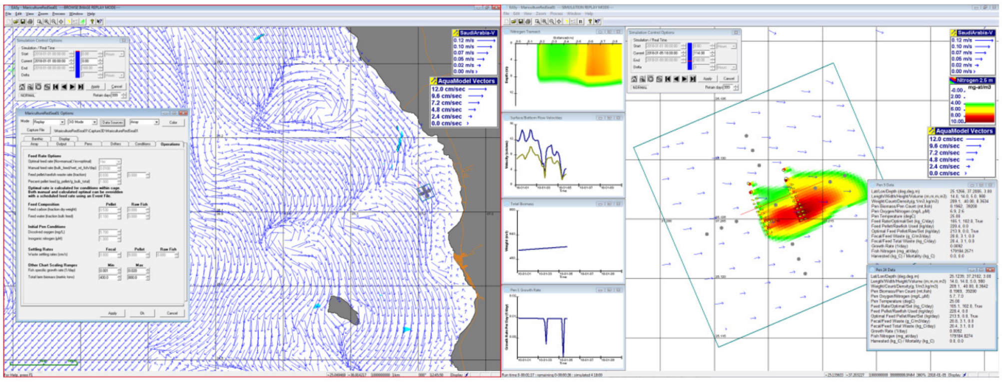


Fig. 1. AquaModel components for finfish aquaculture.



Discussion

AquaModel output enables to determine the environmental pressure for each farm site. This output can be analyzed to suggest sustainable carrying capacity at each of the six sites, and can lead to recommendations for farm operation modification, monitoring and further research.

Conclusions

This exercise allows for the assessment of the carrying capacity of the selected sites, by simply increasing the production level assigned to each farm until the maximum sustainable production is attained, even separating the points of emissions as adding new farms in the selected area. The maximum carrying capacity is highlighted by the interference of the nitrogen plumes with surrounding coral reefs over space and time dimensions.

35 YEARS OF CONSERVATION OF THE ADRIATIC STURGEON: PAST AND FUTURE

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The Adriatic sturgeon (*Acipenser naccarii*), endemic of the northern Adriatic Sea and its main tributaries, is the only one still surviving of the three sturgeon species once living sympatrically in Italy. The other two formerly present species were the common sturgeon (*A. sturio*), distributed also along all the Italian coasts and the Beluga, (*Huso huso*).

The survival of the Adriatic sturgeon has been possible thanks to a variety of conservation actions conducted in the last almost 35 years by different public and private entities, but, most importantly, were possible only thanks to the efforts of a private trout farmer who, anticipating the extinction of this species, collected and maintained in his fish farm about 90 animals of wild origin caught in the Po and other northern rivers of the Padana Valley. From this stock, since 1988 hundreds of thousands of animals have been produced through controlled ex situ reproduction and were mostly released in the wild and in part retained as future breeders.

The different conservation activities carried out in Italy have been crucial for the preservation of the species, in spite of the lack of global coordination that led to a suboptimal management of the residual population at different levels: i) choice of individuals to reproduce to maximize genetic diversity, ii) establishment of training and releasing protocols to maximize the fitness for survival of the reintroduced animals, iii) organization of an effective and coordinated monitoring activity.

The present report will trace the whole history of the conservation of the Adriatic sturgeon in Italy, combining the main actions carried out into a single picture, from the beginning until the recent findings of some mature female and fingerlings and juveniles in some rivers, most probably the results of some natural reproductions. Furthermore, a huge and ambitious project for the establishment of a center for the conservation of freshwater biodiversity of the Padana Valley, presently in progress, will be presented.

FIRST COMPARATIVE EVALUATION OF *Ostrea edulis* (LINNAEUS, 1758) AND *Crassostrea gigas* (THUNBERG, 1793) BIODEPOSITION IN TWO MEDITERRANEAN LAGOONS

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Introduction

Oyster farming represents 33% of global bivalve production. The Pacific cupped oyster *Crassostrea gigas* (Thunberg, 1793) is the most reared species (~653,000 tons in 2019), while the production of the European flat oyster *Ostrea edulis* (Linnaeus, 1758) is less substantial (~2,200 tons in 2019) (FAO, 2019). Oyster farming is associated to some important ecosystem services (Clements and Corneau, 2019), but simultaneously it could negatively impact on the sea bottom health, through the release of faeces and pseudofaeces, rich in inorganic and organic matter (Newell, 2004), hereafter collectively referred as bio-deposits. High amounts of bio-deposit production can cause structural changes in benthonic communities, leading to hypoxic events, loss of biodiversity and predominance of detritivorous species (Newell, 2004). Eutrophic environments such as coastal lagoons are characterised by large amount of phytoplankton and suspended particulate matter available for filter-feeders, thus representing suitable sites for oyster farming but also resulting in a large production of bio-deposits (Newell, 2004). The aim of this study was to compare the daily bio-deposition rate of two oyster species, *C. gigas* and *O. edulis*, in two coastal lagoons potentially suitable for farming and characterised by different environmental and trophic conditions.

Materials and methods

This study was conducted in two shallow eutrophic lagoons (Magni et al., 2008) located in the central-western coast of Sardinia Island (Western Mediterranean, Italy), S'Ena Arrubia and Corru S'Ittiri. In September 2021 and over a period of 24 hrs, we evaluated the bio-deposit production of the two oyster species and the natural deposition rate of the lagoons: Total Particulate Matter (TPM), Particulate Inorganic Matter (PIM) and Particulate Organic Matter (POM). Seven replicates per treatment were performed. Individuals had a mean wet weight of 43.4 ± 0.8 g (*C. gigas*) and 46.3 ± 0.6 g (*O. edulis*), they were of hatchery origin (Marinove, France) and they were reared in the same lagoons. Prior to the trials, individuals were brushed cleaned to remove epibionts and acclimated for a week to the natural conditions of the lagoons. The bio-deposit production and the natural deposition were evaluated by using 21 (7 per treatment) cylindrical PVC tubes with a close-end fund (200 mm, height; 100 mm, diameter), suspended on a floating rope at 15 cm depth. Environmental parameters (temperature, salinity and dissolved oxygen) were measured with data loggers placed at 15 cm depth. Trophic conditions were assessed on surficial water samples collected in triplicates for measurement of chlorophyll a (Chl-a), TPM, PIM and POM, following the methods described in Parson, Maita & Lalli (1984). Data were analyzed by Statistica 6.1 StatSoft, Inc. (2004).

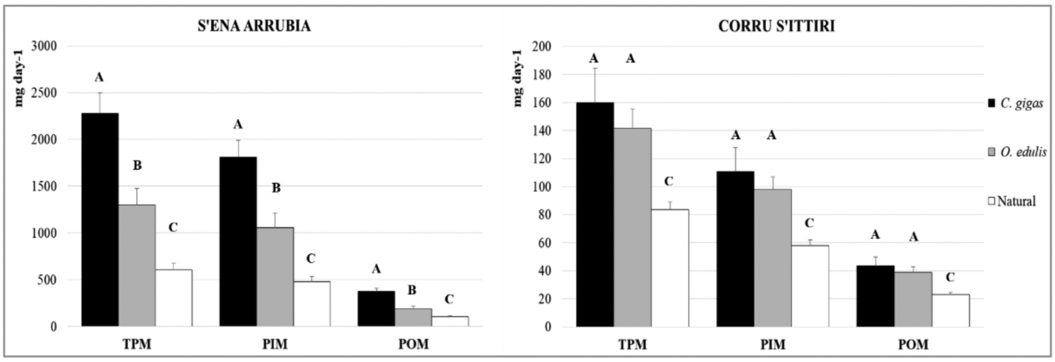

Fig. 1: TPM, PIM and POM production of *C. gigas*, *O. edulis* and Natural sedimentation in Corru S'Ittiri e S'Ena Arrubia. Superscripts indicate significant differences among treatments (n=7).

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Results

In S'Ena Arrubia, the bio-deposit production was significantly different between species, *C. gigas* showed higher production of TPM ($p < 0.01$), PIM ($p < 0.01$) and POM ($p < 0.001$) than *O. edulis*. In Corru S'Ittiri, although we registered high values of TPM, PIM and POM produced by *C. gigas*, no significant differences were highlighted in comparison with *O. edulis*. Natural sedimentation resulted significantly different between lagoons, with a higher production of TPM, PIM and POM in S'Ena Arrubia than Corru S'Ittiri ($p < 0.01$) (Fig. 1). Environmental and trophic parameters were significantly different between S'Ena Arrubia and Corru S'Ittiri. In particular, Corru S'Ittiri registered a higher salinity ($p < 0.05$) and Chl-a ($p < 0.001$), and a lower amount of TPM ($p < 0.001$) and PIM ($p < 0.001$) than S'Ena Arrubia.

Discussion and conclusions

According to our results, the bio-deposit production of oysters was different between species and varied on the basis of the farming site characteristics. In the two lagoons the amount of bio-deposit produced by *C. gigas* was higher than those produced by *O. edulis*, this probably due to a higher filtration activity and metabolic rate of *C. gigas*, as previously observed by Nielsen et al. (2017) under laboratory conditions. Regardless of the oyster species we recorded a higher bio-deposit production in S'Ena Arrubia, probably due to the higher amount of TPM and PIM in the water column in comparison with Corru S'Ittiri, rather than the amount of Chl-a, which was higher in Corru S'Ittiri. In conclusion, the two oyster species produce different amount of bio-deposit, the production is site-specific and vary on the basis of the farming site characteristics, in particular the amount of available particulate matter. This indicates that the effects of oyster aquaculture on the sediments of the farming site must be evaluated specifically in each location.

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A COMPARISON BETWEEN HYDROPONIC AND AQUAPONIC GROWN SALANOVA LETTUCE INFECTED BY *Pythium* sp.

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Introduction

Soil-independent, closed environment agriculture (CEA) has potential to meet challenges in food production, such as climate change and soil deterioration by assuring yield, improving water and fertiliser efficiency, decreasing the incidence of pathogens and pests, among others. The use of recovered or recycled fertilisers instead of chemically pure mineral fertilizers would further contribute towards increasing the sustainability of vegetable production. By combining hydroponics (HP) with aquaculture, nutrient-rich aquaculture effluent is recirculated, thereby diminishing or removing the need for inorganic fertilisers. As additional benefit, cultivation in aquaponics seems to provide competitive presence of beneficial microorganisms that contribute towards mitigation of root rot infections1–3. However, detailed studies of this are rare. Pythium spp. are root pathogens that often cause root rot in soil-less grown vegetables. To test the hypothesis that aquaculture effluent contributes toward fighting against Pythium spp. infection, lettuce (Lactuca sativa, variety Salanova) was cultivated in either conventional HP or in the effluent of a commercial aquaculture farm before being inoculated with the pathogen.

Materials and Methods

Twenty-four lettuces were grown in rafts in a climate chamber (23-15 °C day/night, 65% relative humidity, 16-8h day/night, 0.12% CO_2) and underwent two treatments in duplicate boxes of 6 plants each: *(i)* HP solution of demineralised water with added nutrients and NaCl to mimic the composition of the aquaculture effluent; *(ii)* aquaculture effluent from a commercial RAS rearing pike perch at 37 kg/m^3 (AP). AP contained 782 mg/L Na^+ and 830 mg/L Cl^- (4.2 mS/cm) as the farm used NaCl to prevent disease in fish. K and Fe were added in AP as KOH and Fe EDTA, respectively, to meet lettuce requirements. pH at the end of the trial was 7.95 and 7.88 in HP and AP, respectively. On day 29 after seeding, 0.25 g *Pythium* sp.-infected millet was inoculated onto each rockwool cube of one replicate. After 60 days from seeding, chlorophyll, flavonoid, and nitrogen levels were estimated with Dualex® (ForceA, France)[4]. On day 61, the lettuces were harvested, shoots and roots were weighed separately and thereafter dried at 60 °C before determining dry matter. The elemental composition was analysed by XRF spectrometry (SPECTRO analytical instruments GmbH, Germany). R software[5] was used to perform a two-way ANOVA with factors system (S; levels: HP, AP) and infection (I; levels: no, yes), followed by a Tukey's test.

Results and Discussion

All parameters, except shoot dry weight, root dry weight, and nitrogen balance index, differed between HP and AP systems ($p<0.05$ or smaller; Table 1). Plant growth in the AP system was lower than in the HP system, as found elsewhere[6]making it difficult to accurately determine deficient nutrients limiting crop yield and quality across the systems. To avoid interference with background nutrients, we used reverse osmosis water in this study. The objectives were to identify critical nutrients that affect the yield and quality of cherry tomato-, basil-, and lettuce by characterizing nutrient composition and concentration in aquaponic systems in comparison to hydroponic systems. Daily release rate (mg L−1. There were no visible signs of infection on lettuces from either system. AP plants undergoing *Pythium* infection did not show differences from the non-infected ones, while the percentage of shoot dry weight on fresh weight in infected HP-grown lettuce was significantly higher. Infecting the plants also led to a not significant increase in the levels of flavonoids (stress response) and chlorophyll (possibly related to nitrogen status[7]), irrespective of the system. However, the nitrogen balance index was not significantly different, indicating that the nitrogen status of leaves was not sharply affected.

Na, Ca, Mg, P, Cl, Mn, Cu and Zn contents were significantly higher in shoots of HP-grown plants, while there were no differences in K, Al, and Si; Fe and Ni were higher in the shoots of AP-grown plants (Table 2). The levels of Fe in shoot biomass seemed to be lower in the infected plants. The lack of replicates did not allow to detect statistical differences between the different treatments in the element concentrations in root samples.

Conclusions

The study showed that HP and AP nutrient solutions containing similar values of pH, nutrients and salinity could both sustain Salanova lettuce growth. Infection with *Pythium* sp. did not compromise the lettuce growth; however, the beneficial effect could not be confirmed. As there were no visible signs of infection on lettuces from either system, *inocula* containing more active spores should be tested to delve into the capacity of AP system to provide beneficial bacteria against pathogens.

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Table 1. Lettuce growth (means from 3 pools of 2 lettuce per treatment), and estimates of leaf chlorophyll, flavonoid and nitrogen balance index at harvest measured by Dualex® (ForceA, France) (means from 4 leaves of 3 lettuce per treatment).

System (S)	**HP**		**AP**		**ANOVA results**	**SEM**
Infection (I)	**No**	**Yes**	**No**	**Yes**		
Shoot fw [g]	179.8^{a}	168.0^{a}	97.5^{b}	137.0^{ab}	S: **	5.9
Shoot dw [g]	8.5	10.9	9.0	11.5	I: *	0.3
Root fw [g]	49.5^{a}	41.6^{a}	15.2^{b}	19.3^{b}	S: ***; S×I: *	2.3
Root dw [g]	3.0^{a}	2.5^{a}	0.9^{b}	1.2^{b}	S: ***	0.1
Chlorophyll	22.99^{b}	24.38^{ab}	27.58^{ab}	30.96^{a}	S: **	0.996
Flavonoid	0.075	0.159	0.222	0.292	S: *	0.019
Nitrogen balance index	217.0	282.6	214. 0	127.0	ns	27.5

*SEM: standard error of the mean. fw: fresh weight; dw: dry weight. Different superscripts indicate: *: p<0.05; **: p<0.01; ***: p<0.001. ns: no statistically significant difference.*

Table 2. Average lettuce element concentrations (on dry weight) in the shoots at harvest; values are means from 3 pools of 2 lettuce per treatment.

System (S)	**HP**		**AP**		**ANOVA results**	**SEM**
Infection (I)	**No**	**Yes**	**No**	**Yes**		
Na [mg/g]	51.92^{a}	42.59^{b}	32.85^{c}	33.85^{c}	S: ***; I: **; S×I: **	1.18
Ca [mg/g]	14.06^{a}	9.94^{b}	7.06^{c}	7.10^{c}	S: ***; I: ***; S×I: ***	0.44
Mg [mg/g]	5.43^{a}	4.26^{b}	2.86^{c}	2.72^{c}	S: ***; I: **; S×I: **	0.17
P [mg/g]	9.77^{a}	7.35^{b}	5.39^{c}	5.26^{c}	S: ***; I: **; S×I: *	0.29
Cl [mg/g]	32.79^{a}	27.79^{b}	19.15^{c}	19.64^{c}	S: ***; I: *; S×I: *	0.88
Fe [μg/g]	80.8	69.2	113.6	96.1	S: *	3.4

*SEM: standard error of the mean. Different superscripts indicate significant differences for the system factor: *: p<0.05; **: p<0.01; ***: p<0.001. ns: no statistically significant difference.*

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FIRST SUCCESFUL ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) FEEDING DEMONSTRATION WITH FORMULATED DIETS: THE ONLY SUSTAINABLE FUTURE FOR TUNA FARMING

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Introduction

The tuna ranching industry is a global, high-value aquaculture activity. Current feeding practices are impractical, unsustainable and pose serious ecological risks. The daily feeding of large quantities of fresh/frozen fish results in extremely high feed conversion rates (>20 : 1) and significant nutrient leeching (1, 3, 4, 7, 8). Seasonal availability and quality variation plus the absolute dependence on exotic baitfish brought to the Mediterranean Sea highlight the urgent need for a balanced feed. Objectives for the present experiment were to compare performance and quality indicators of Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) fed herring/mackerel or a compound tuna feed based on sustainable American soy.

Materials and methods

A fourteen-week feeding demonstration was conducted in two oceanic net pens moored east of San Pedro del Pinatar, Murcia, Spain (37°49.835' N 000°39.822' W). Each cage held ~45 fish, weighing approximately 99.5 kg (average initial individual weight, evaluated both by expert visual ranking and corroborated by the AQ1's AM 100 fish sizing system). Feed consumption and condition factor were recorded. At harvest, dorsal loins were collected and color, mercury, proximate composition, scombrotoxin levels, oxidative stability index and peroxide values were evaluated in the resulting tuna steaks. In addition, a blind sensory evaluation and a commercial taste assessment were performed on the main sashimi cuts obtained from fish fed either diet.

Results

Steak samples from formula-fed fish had similar lipid contents as those fed baitfish but exhibited improved flesh color, texture and had increased oxidative stability and reduced histamine. Results from sensory and organoleptic evaluations (professional sashimi chefs) indicated that sashimi slices from formula fed fish were similar in flavor but more stable on the counter and superior in color. Importantly, there was a significant reduction in tissue mercury in formula-fed fish as compared to baitfish fed ABFT (6).

In addition, the formulated diet offered feed management options that are quite compatible with the present operation and equipment available at commercial tuna farms in the Mediterranean Sea (2, 5). A preliminary economic evaluation (2021 prices) indicated that tuna feed is significantly more cost-effective than imported frozen baitfish.

Discussion and conclusions

Given increased fishing quotas for ABFT and the severe difficulties in sourcing baitfish experienced by Mediterranean tuna farmers, the formulated diet offer a viable alternative to baitfish feeding. In conclusion, these results indicate that the balanced feed provided suitable nutrition for adult ABFT, enhanced the quality and shelf life of tuna flesh, and may enable management strategies to optimize performance, thus increasing farm efficiency and reducing the environmental impact of commercial tuna ranching. This research was supported in part by the United States Soybean Export Council.

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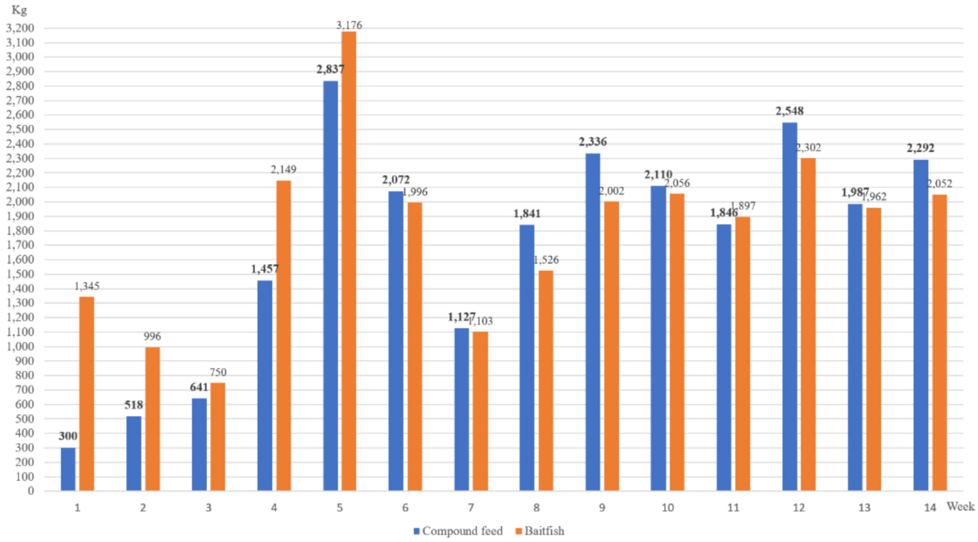
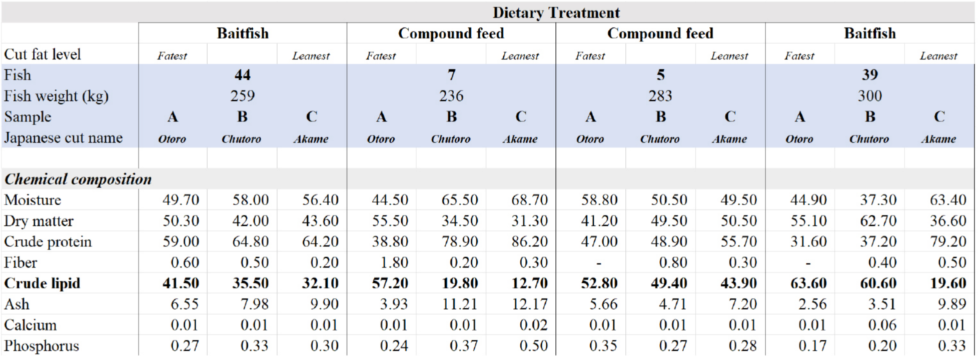


Weekly feed consumption of ABFT (Sep – Dec, 2021). Blue bars = compound feed. Orange bars = baitfish



Dietary Treatment												
	Baitfish			**Compound feed**			**Compound feed**			**Baitfish**		
Cut fat level	*Fatest*		*Leanest*	*Fatest*		*Leanest*	*Fatest*		*Leanest*	*Fatest*		*Leanest*
Fish		**44**			**7**			**5**			**39**	
Fish weight (kg)		259			236			283			300	
Sample	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Japanese cut name	***Otoro***	***Chutoro***	***Akame***	***Otoro***	***Chutoro***	***Akame***	***Otoro***	***Chutoro***	***Akame***	***Otoro***	***Chutoro***	***Akame***
Chemical composition												
Moisture	49.70	58.00	56.40	44.50	65.50	68.70	58.80	50.50	49.50	44.90	37.30	63.40
Dry matter	50.30	42.00	43.60	55.50	34.50	31.30	41.20	49.50	50.50	55.10	62.70	36.60
Crude protein	59.00	64.80	64.20	38.80	78.90	86.20	47.00	48.90	55.70	31.60	37.20	79.20
Fiber	0.60	0.50	0.20	1.80	0.20	0.30	-	0.80	0.30	-	0.40	0.50
Crude lipid	**41.50**	**35.50**	**32.10**	**57.20**	**19.80**	**12.70**	**52.80**	**49.40**	**43.90**	**63.60**	**60.60**	**19.60**
Ash	6.55	7.98	9.90	3.93	11.21	12.17	5.66	4.71	7.20	2.56	3.51	9.89
Calcium	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.06	0.01
Phosphorus	0.27	0.33	0.30	0.24	0.37	0.50	0.35	0.27	0.28	0.17	0.20	0.33

Proximate composition of sampled tuna tissues

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MUSSEL TRANSMISSIBLE NEOPLASIA MtrBTN: WHEN CANCEROUS CELLS ADOPT A PARASITIC LIFESTYLE

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Introduction

After accumulating mutations, cancers diverge from the host becoming a new organism inside it, unable to survive autonomously. However, the parasitic status of cancers could be disputed on this basis —they are non-contagious. But some cancers evolved the ability to transmit from host to host (Dujon et al., 2020). They spread by transmission of living malignant cells becoming atypical parasitic life forms: parasites with a host-related genome. The transmissibility of these cancerous cell lines has been demonstrated through i) the presence of genetic chimerism in the diseased animals, with genetic differences between tumor and host cells, (ii) the genetic similarity between tumors of different individuals, iii) successful experimental transmission. Several transmissible cancers have been described, most in bivalves, including farmed marine mussels. MtrBTN2 lineage has been detected worldwide in various *Mytilus* species (Burioli et al., 2019; Hammel et al., 2021; Skazina et al., 2021; Yonemistu et al., 2019), crossing the species barrier. We accumulated data on epidemiology, genetics and phenotypic traits of MtrBTN2, focusing on those reminiscent of a parasitic lifestyle.

Material and methods

Between 2017-2022, we collected over 5000 mussels of the two species farmed in France (*M. edulis* and *M. galloprovincialis*), in 10 sites along the French coasts. Samplings were carried out on farmed and wild batches. We used cytology, FACS and genetics to diagnose MtrBTN2. We investigated the potential effect of size/age, sex, environment type, season, and presence of other co-factors (such as other parasites) on prevalence. Characterization of survival capacities in the outside-host environment is lacking for MtrBTN2 cells unambiguously demonstrated to be transmissible. We assessed the survival capacity of cancerous cells in seawater (SYTOX). Host colonization success depends also on the ability of parasites to proliferate in their hosts. Thus, we evaluated in-host proliferation rates by performing EdU incorporation assays. As a consequence of their lifestyle, parasites often have profound effect on the fitness of their hosts. When parasites harbor significant multiplication rates, the intra-host load that can be reached depends strongly on the host resources available for parasite population growth (i.e. host carrying capacity). An increased host carrying capacity can be achieved by manipulating host energetic resources to the parasite profit, for instance through host castration. Thus, we investigated the cost of MtrBTN2 infection on the host fecundity. We determined the reproductive condition by histology.

Results and discussion

We observed a quite low prevalence in all sites (0% to 25%), with significant differences among them, and rather constant along seasons. No correlation was noticed with host size/age, sex, and presence of parasites. However, we observed a strong species-related sensitivity, higher in *M. edulis*. We evidenced the coexistence of two MtrBTN2 sub-lineages that we characterized genetically and phenotypically. They differ highly on their DNA content (about 10N and 17N).

MtrBTN2 cells survival rate in seawater (Fig. 1) was significantly higher than that of hemocytes over 7 days with a median survival time of MtrBTN2 cells equal to 6 days, as opposed to 4 days only for hemocytes. With almost 100% cell survival over three days, chances to infect neighboring hosts are high. MtrBTN2 cells were also characterized by a high proliferation capacity, more than 17 times higher than that of normal hemocytes in healthy mussels. They favor a rapid increase of intra-host population size (mean doubling time of~3 days). Finally, MtrBTN2 appears to induce host castration (Fig. 2), favoring resources re-allocation and increasing the host carrying capacity.

Altogether, our results indicate that MtrBTN2 cells behave like unicellular castrator parasites.

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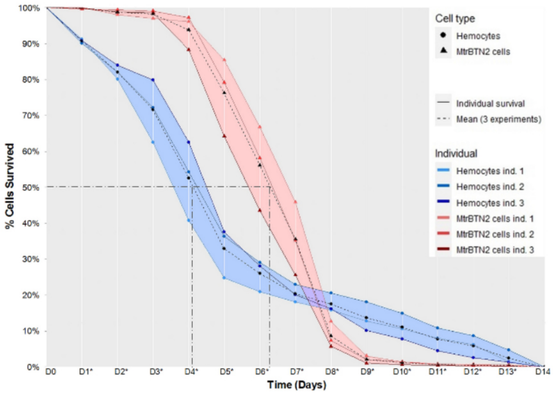

Fig. 1: MtrBTN2 cells survive longer than hemocytes in seawater. Cells were incubated at 18 °C for 14 days. Dotted lines are the means of three independent experiments from different individuals; solid lines are the means of the three wells from each individual.

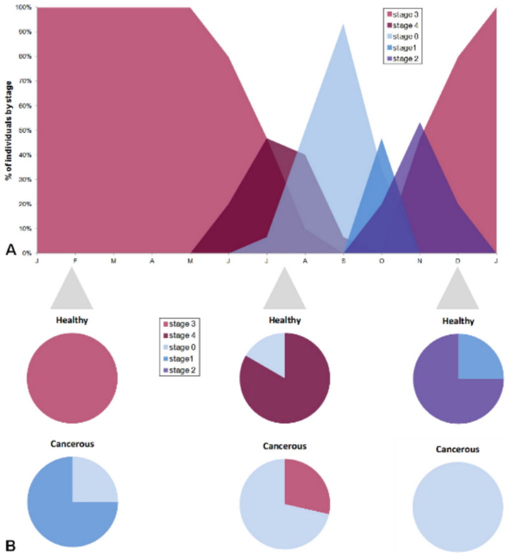

Fig. 2: Gonadal development is delayed in MtrBTN2-affected mussels. (A) reference study in healthy mussels; (B) seasonal results from the present study comparing healthy and cancerous mussels. Stage 0: resting; stage 1, gonia proliferation; stage 2: all developmental stages present with only few mature gametes; stage 3: predominance of mature gametes; stage 4: resorption.

TEMPORAL AND SPATIAL VARIABILITY IN TEMPERATURE AND OXYGEN ALONG SOUTHWEST NOVA SCOTIA USING GLIDER OBSERVATIONS

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Introduction

Climate change has threatened coastal ecosystems through elevated sea temperatures combined with increased nutrients through expanding development. Both warmer waters and higher nutrient loads have resulted in intensification of coastal oxygen deficits (Breitburg et al. 2018). This loss in oxygen can impact ecosystem functioning, degrading coastal habitats, and threatening the aquaculture industry that are often established in these areas. Previous studies have documented temperature and salinity variability across the Scotian Shelf (Nova Scotia, Canada), however oxygen distribution has not been as well characterized (e.g. Dever et al. 2016). This study explores the along-shore and cross-shore variability in temperature, salinity, and most importantly dissolved oxygen, while also exploring the interaction between offshore observations and those within a bay suitable for aquaculture farms. The inner Scotian Shelf and bays are intrinsically linked thus it is important to understand the oxygen dynamics between them, ultimately allowing for better farm management.

Materials and methods

A Slocum underwater profiling glider was deployed between September 25 and October 12 2020 along the inner Scotian Shelf between Shelburne Bay and St. Margarets Bay. A novel zigzagging method was used to collect high-resolution temperature, salinity and dissolved oxygen data and capture both along-shore and cross-shore variability. Additionally, a real-time optical sensor was deployed within St. Margarets Bay to record temperature and dissolved oxygen for comparisons with the offshore glider data.

Results

The along-shore transect was characterized by strong vertical stratification in temperature and oxygen between September 26 and October 9. This was followed by a strong vertical mixing event, resulting in a collapse of the thermocline and cooling of the water column from 15.5 ± 0.73 °C (mean ± SD) to 5.35 ± 1.14 °C. This mixing event lasted for about 11 h before the thermocline was re-established. The cross-shore transects depicted more variability below the mixed layer with distinguished inner and outer profiles with respect to the coast. The inner profiles were warmer (8.32°C inner and 6.42°C outer), fresher (31.3 inner and 31.6 outer profiles) and less oxygenated (8.93 mg L^{-1} inner and 9.32 mg L^{-1} outer profiles). Lastly, the inshore sensor located within St. Margarets Bay captured the vertical mixing event 30 h after the glider observations, indicating the upwelled waters from further offshore likely advected inshore resulting in decreased temperatures, and increased oxygen, observed in the Bay.

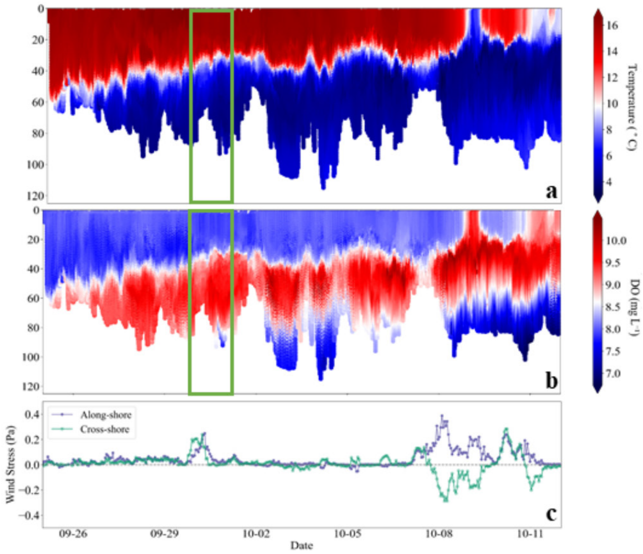

Figure 1. (a) The vertical distribution of temperature (°C; b), and dissolved oxygen (mg L^{-1}; b), along the Slocum glider transect. The glider was programmed to turn 2 m above the seafloor. (c) The along-shore (U, m s^{-1}; purple) and cross-shore (V, m s^{-1}; teal) wind stress components.

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Discussion

Strong along-shore winds to the northeast result in surface waters moving offshore and subsequent replacement with cool, deep waters, ultimately leading to the cooling of the upper water column observed on October 9. This mechanism follows the expected response due to Ekman transport for Nova Scotia waters (Shan and Sheng 2022). Cross-shelf vertical structure has been observed along the Halifax line (Dever et al. 2016), however the method employed in the present study is novel and allowed for multiple cross-shore observations along the inner shelf-coast of southwest Nova Scotia. This demonstrates the efficiency of glider observations for temporal, alongshore and cross-shore variability of hydrography and DO concentration over the inner Scotian shelf. Higher DO was associated with lower temperatures observed in the outer region of the transect due to increased solubility, though the glider may have also intercepted a water mass with differing properties to those more inshore. Lastly, this study showed how an increase in oxygen due to upwelling along the shelf can be horizontally advected towards the shore, ultimately allowing for increased oxygen concentrations around potential aquaculture farms. Having access to high spatial and temporal resolution around bays will help to quantify the role that the coastal shelf may play in oxygen dynamics within the bay, ultimately leading to improved aquaculture farm management.

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THE STURGEON BY-CATCH WHILE TRAWL FISHING IN THE NORTH-WESTERN PART OF THE BLACK SEA

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Introduction

Observations on sturgeon by-catch during sprat trawl fishing operations in the Black Sea were conducted in framework of Discard monitoring program as part of the GFCM project "Select Activities Implemented of the Mid-term Strategy towards the Sustainability of Mediterranean and Black Sea Fisheries" (BlackSea4Fish). Studies were carried out by observers aboard of Ukrainian SChS and PTR-type vessels (about 25 m length). The study period lasted from August to November in 2018, from April to November in 2019 and in August-September 2021.

Materials and methods

Observations were performed according to the methods described in the FAO manual (FAO, 2019). The sprat fishery was conducted in the north-western Black Sea in the area bounded by longitude 31.3200 E from the east, latitude 46.0000 N from the south, and the coastline from the north and west. The trawling operations were carried out using midwater trawl in the near-bottom layer at the depths of 9 to 33 m at a distance of 1.5 to 50 km from the coast. The vessel speed during tows was 3.5–4.0 knots. All fishing operations were conducted during daylight hours. The duration of one trawling operation varied from 0.5 to 3–4 hours. The sprat trawl fishery in the NWBS is characterized by high selectivity. The proportion of all other fish species in catches (whiting, anchovy, sturgeons, turbot, sand smelt, shad, horse mackerel, etc.) did not usually exceed 1.0–1.5%.

The catch composition was studied during 342 fishing operations (see Table 1). The average sprat catch per hour of trawling (catch per unit effort, CPUE) was 0.57 ton in 2018; 0.44 ton in 2019 and 0.85 ton in 2021.

Results and discussion

The most undesirable phenomenon registered during the period of observations was the by-catch of young sturgeons - the most "vulnerable" group of fish in the Black Sea. The Starry sturgeon *Acipenser stellatus* (Pallas, 1771) and the Russian sturgeon *Acipenser gueldenstaedtii* (Brandt & Ratzeburg, 1833) were quite regularly presented in mid-water trawls in 2018-2019. Beluga *Huso huso* (L., 1758) was also caught in 2021 and even in greater numbers than Russian sturgeon. In 2018-2019, sturgeons were recorded in 50 trawls out of 283 (approximately in one haul out of six). And in 2021, they were registered in 52 hauls out of 59, that is, in almost every trawl.

Individuals of Starry sturgeon absolutely dominated among all sturgeons in trawl by-catches. Their share was 89.7% in 2018, 98% in 2019 and 93.8% in 2021. The share of Russian sturgeon in this by-catches was 5.1% in 2018-2019 and 2.8% in 2021. The share of beluga was 3.4% in 2021.

All sturgeon species in the by-catch were represented mainly by young individuals, which were characterized by relatively small size and weight (Table 2).

Only one Russian sturgeon and two Starry sturgeons had a body length of more than 1 m and could potentially reach sexual maturity among all the sturgeons caught in 2018-2019. The number of large individuals in the by-catch slightly increased in 2021: Russian sturgeon – 2 ind., starry sturgeon - 13 ind. Among 10 belugas caught in 2021 two had a body length exceeding 2 m, which allows them to be classified as sexually mature. Thus, we can state a slight upward trend in the size and weight characteristics of sturgeons over the past two years. The extremely low proportion of adult fish in this by-catch can be explained by their elimination by illegal fishing in the Danube and Dnieper rivers and at sea.

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Table1. Fishing efforts and by-catch of sturgeons in the NWBS in 2018-2019 and 2021

Year	Month	N of hauls	Sprat catch, t	*A. stellatus*		*A. gueldenst.*		*H. huso*	
				n	m, kg	n	m, kg	n	m, kg
2018-2019	VIII-XI, IV-XI	283	278	74	120	4	16	-	-
2021	VIII-IX	59	91	272	502	8	53	10	394

Table2. Size-weight characteristics of sturgeons in trawl by-catch.

Year	*A. stellatus*		*A. gueldenstaedtii*		*H. huso*	
	l, cm	m, kg	*l*, cm	m, kg	*l*, cm	m, kg
2018-2019	32 -102	0.2 – 4.7	42 - 102	0.5 – 9.6	-	-
2021	40 -109	0.3 – 6.3	60 - 112	2.2– 13.0	98 - 256	9 -150

Proportion of sturgeons among all by-catch fish weight amounted to 61.2% in 2021. The number of sturgeons caught per trawl operation has increased significantly over the past two years. It amounted to 0.24 ind. in 2018, 0.28 ind. in 2019, and already 4.91 ind. in 2021. If in 2018 the by-catch of one individual of sturgeons accounted for 5.8 tons of sprat, in 2019 - for 2.3 tons, then in 2021 - only for 1.54 tons of sprat. So the fact of a noticeable increase in the number of at least one species - starry sturgeon in recent years is difficult to deny.

Obviously, the observed recovery of the Starry sturgeon population in the NW Black Sea was made possible by relatively successful natural spawning in the Danube River. Beside it can be assumed that the growth of sturgeon populations has been facilitated by restrictions on fishing in this marine area provided by the Russian Navy.

Some special study was conducted to search for CWT tags using T-wand detector to assess the role of artificial sturgeon stocking carried out massively in Romania with tagging of all individuals released in 2008-2015. 69 Starry sturgeons, 4 Russian sturgeons and 3 belugas were examined in trawl by-catches. The presence of tags was detected for two individuals of the Russian sturgeon only. Nothing tags were found in individuals of the other two species. This fact may indicate that the rate of the natural spawning of Russian sturgeon in the Black Sea basin is extremely low, and artificial stocking of this species can significantly help to preserve its population.

EFFECTS OF SCP FROM *Candida utilis* ON GROWTH PERFORMANCE, PLASMA BIOCHEMISTRY, GUT MICROBIOTA, AND IMMUNE RESPONSE OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

The increasing global need to find alternative and sustainable protein sources has promoted research in the field for non-conventional feed ingredients, such as single-cell proteins (SCPs). SCPs production is based on the fermentation of microorganisms like yeast, bacteria, microalgae, and filamentous fungi. Many of these species have high protein content, and some have amino acid profiles like that of fishmeal (FM)[1]. The studies conducted on yeast SCPs indicated that dietary inclusion of up to 30% improves feed efficiency[2,3], and can reduce inflammatory processes in fish[4]. To date, a variety of substrates have been utilized to cultivate microorganisms for the purpose of SCP production, such as agricultural wastes, fruit processing wastes, methane, and animal wastes[5]. This study was undertaken to assess the effects of different inclusion levels of SCPs on growth, feed utilization, fish plasma biochemistry, immune response, and gut microbiota of gilthead seabream reared under normal and after suboptimal conditions. The product utilized in this trial is a dried, inactive *Candida utilis* produced from sustainable feedstocks to partly or fully replace traditional protein sources in fish feed.

Materials and methods

One trial with gilthead sea bream was conducted in a closed recirculation aquaculture system (RAS). Fish (initial weight: 24.8 ± 0.1g), were fed over 76 days with four experimental diets containing different inclusion levels of SCPs meal (0% CTRL, 5% SCP5, 7.5% SCP7.5, and 10% SCP10) in substitution to FM. After the end of the trial, fish were subjected to suboptimal rearing conditions (high water temperature, 30°C, and low oxygen, 70% saturation level) for 8 days. Growth and feed efficiency parameters (specific growth rate, SGR, feed intake, FI, feed conversion rate, FCR), blood biochemistry, immune response marker genes of the kidney and distal intestine (Interleukin 1 β, Interleukin 8, Interleukin 10, Interleukin 6, Transforming growth factor β, Tumor necrosis factor α), and gut microbiota were assessed at the end of the trial and after suboptimal rearing conditions. Data were analyzed by a one-way ANOVA followed by Tukey's multiple comparison test.

Results

At the end of the trial, no significant differences ($p > 0.05$) due to the different treatments were observed in FI, while significant differences ($p > 0.05$) were observed in final body weight, SGR, and FCR (Figure 1).

Discussion and Conclusion

The results show that experimental diets containing different levels of SCPs inclusion from *Candida utilis* led to statistically significant differences in growth performance. In particular, differences were found in the average final weight and in the SGR and FCR indices, from which it emerged that the 7.5% inclusion of SCP is more beneficial to the growth performance of the animals.

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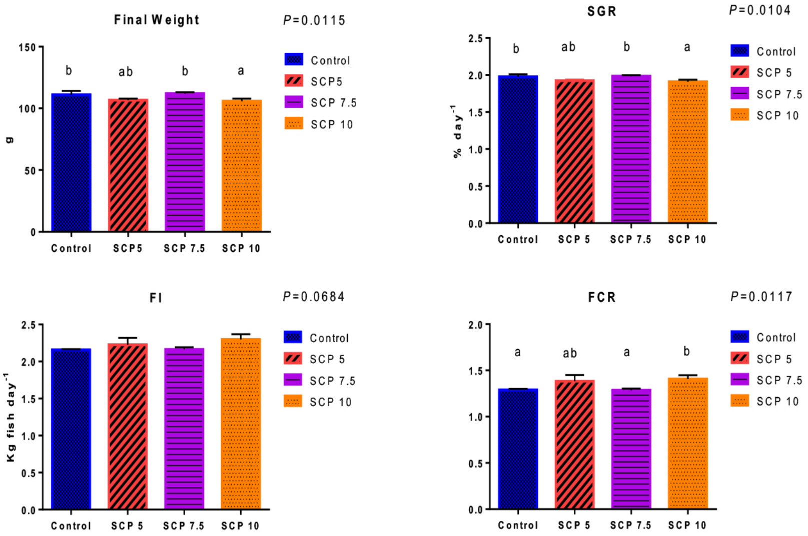

Fig.1. Final body weight, SGR, FI, and FCR obtained at the end of the trial under different treatments. Different superscript letters a, and b denote significant differences ($p < 0.05$) among diets.

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CHEMOSTAT OPERATION AND PRODUCTIVITIES IN A 25 L TUBULAR SYSTEM AT DIFFERENT LIGHT INTENSITIES AND DILUTION RATES

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The production process of complicated and fragile microalgae species including *Rhodomonas* is essential for industrial implementation of microalgae in aquaculture. Using our proprietary Lgem photobioreactor (PBR) systems we are able to cultivate many fragile microalgae species with high efficiency and reliability. Here we present the data on small-industrial scale production of *Rhodomonas sp.* for aquaculture applications. In this presentation we showcase significant improvements in a production process that can be made with the correct tools and a team of experts.

Due to its fragile nature, data for large-scale production of *Rhodomonas* spp. is limited. We investigated the effect of light intensity (124-189 umol m^{-2} s^{-1}) and dilution rate (0.19-0.74 d^{-1}) on the biomass productivity and biomass yield on light, during continuous operation (chemostat) under low-cost light emitting diodes (LEDs) in the Lgem LAB-25 tubular PBR.

A maximum biomass productivity (1.09 g L^{-1} d^{-1}) and biomass yield on supplied light (0.53 g mol^{-1}) was obtained at a dilution rate of $\mu = 0.74$ d^{-1} under high light (189 µmol m^{-2} s^{-1}) compared with 0.72 g L^{-1} d^{-1} and 0.54 g mol^{-1} respectively at a dilution rate of $\mu = 0.42$ d^{-1} under low light (124 µmol m^{-2} s^{-1}). Biomass productivity increased with higher light intensity (except at $\sim\mu = 0.40$ d^{-1}), but was not significantly affected by dilution rates (see Fig. 1a). To reduce the cost of indoor production utilising artificial light, a high efficiency of light use (biomass yield on light) is required. All tested conditions resulted in a high, but not significantly different, biomass yield on light supplied (Fig. 1b). This high yield on light reveals the efficiency of the Lgem PBRs in cultivating fragile microalgae species at pilot scale. Lower dilution rates ($\mu = 0.29$ d^{-1}) result in a 2.3-fold higher biomass concentration (3.42 g L^{-1} DW) compared to high dilution rates ($\mu = 0.74$ d^{-1} at 1.48 g L^{-1} DW) while maintaining similar biomass productivities, resulting in lower water usage and pumping requirements.

The cost of production in aquaculture hatcheries was calculated at ~€286 kg^{-1} at small production scales (25 m^2 scale). By utilising Lgem PBRs the biomass cost can be reduced to <€53 kg^{-1} through the use of artificial illumination at larger production scales (1500 m^2) (Oostlander et al., 2020). The assumptions for the technoeconomic assessment conducted by Oostlander et al. (2020) are taken into account in the current study. Major cost factors are the lighting (investment and operating cost), the efficiency at which this light is used, and the required amount of labour including labour linked to downtime for cleaning. In the presented chemostat study >200 days of operation were achieved without downtime and no fouling was observed whilst maintaining high biomass yield on light under all tested conditions (see Fig. 1b). With Lgem PBRs at large-scale fully automated operation is possible, lowering the requirement for skilled labour in your microalgae production facility.

With *Rhodomonas* sp. as an example we present our reliable and easy-to-use PBR-systems to develop your microalgal production process at our cutting-edge AlgaeHUB®. We have over 15 years of experience in the microalgal industry and specialise in PBR operation and design. In our completely unique 7000 m² facility (near Amsterdam Schiphol airport, The Netherlands), we offer our latest services: lab scale, pilot scale, and commercial scale production systems which are available for testing purposes. Furthermore, we offer various technologies for upstream and downstream processing suitable for fragile microalgae species.

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Results

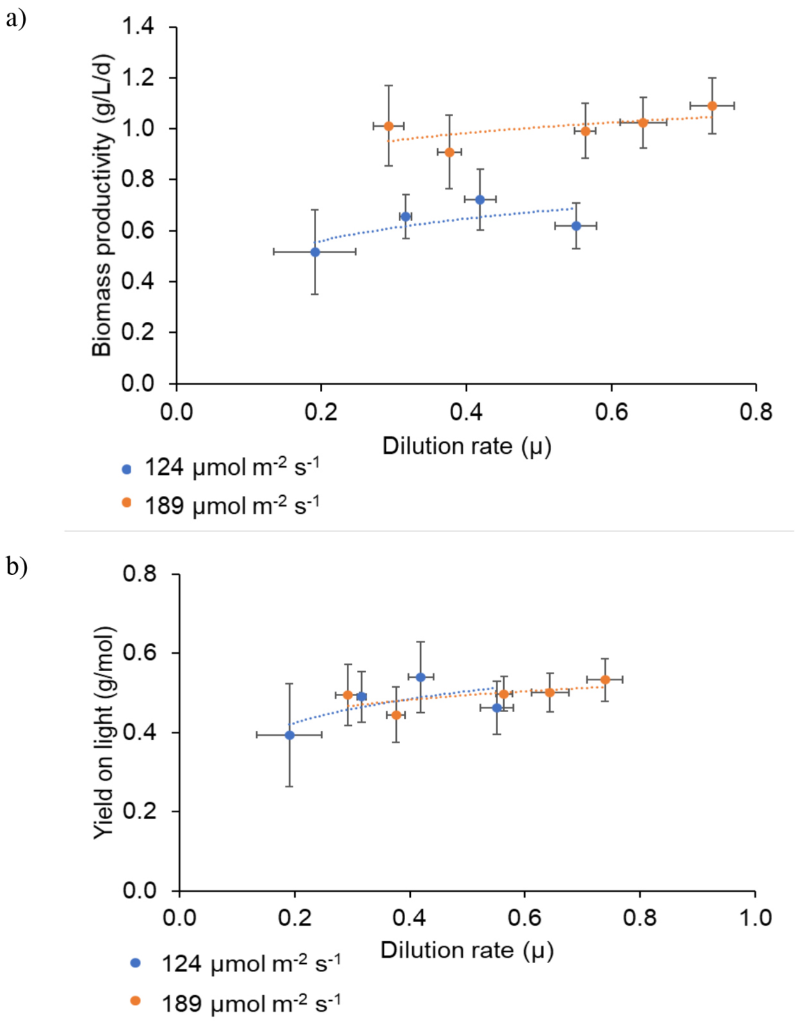

Fig. 1. a) Biomass productivity (g L^{-1} d^{-1}) and b) biomass yield on supplied light (g mol^{-1}) versus dilution rates under chemostat operation in an Lgem LAB-25 tubular PBR (32 mm tube diameter) under red:white light emitting diodes (LEDs) in operation for >200 days. Steady state was defined as at least 3 x culture volumes or a minimum of 9 days for dilution rates <µ = 0.2 d^{-1}. Steady state was obtained when variations in biomass concentration and dilution rate revealed <15 % variation.

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ANTIBACTERIAL ACTIVITY OF *ALLIUM*-DERIVED NATURAL GILTHEAD SEA BREAM PATHOGENS. *IN VITRO* AND *IN VIVO* STUDIES

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Introduction

The use of phytogenics as a method for prevention and control of disease outbreaks in aquaculture has been proposed in recent years [1]. Among the different phytogenics, plant extracts derived from *Allium* spp. such as garlic (*Allium sativum*) and onion (*Allium cepa*), hold a large promise due to their variety of bioactive compounds including polyphenols and organosulfur compounds.

Within the different sulphur compounds present in *Allium* spp., Propyl-Propane-Thiosulfinate (PTS) and Propyl-Propane-Thiosulfonate (PTSO) stand out due to the stability that gives them suitability for their use in feed processes [2] together with multiple functional properties that may contribute to reducing the risk of diseases in fish farming.

In the present study, the *in vitro* antibacterial effect of PTS and PTSO was studied against a number of relevant pathogens for a variety of farmed fish species. Moreover, the effect of both compounds in juvenile gilthead seabream (*Sparus. aurata*) challenged with *Photobacterium damselae* subsp *piscicida* was also evaluated.

Materials and Methods

The *in vitro* antibacterial activity was screened by the disk-diffusion method proposed by Kirby–Bauer [3] and modified by Calvo and Asensio [4]. Sterilized cellulose discs were impregnated with 20 μL of PTS or PTSO at a dose of 2.5, 5, 10, 25, and 50 mg/mL, and placed in the centre of agar plates that were previously inoculated with the target bacterial strains at an adjusted concentration of $1x10^6$ CFU/mL.

The effects of PTS/PTSO on the fish health status were tested in an *in vivo* intraperitoneal (IP) trial against *P. damselae* subsp *piscicida* after 12 weeks of dietary administration of these natural extracts. 90 juveniles gilthead sea bream (*S. aurata*) weighing 4.45 ± 0.39 gr were divided into 2 groups in triplicate after acclimatization at 21 ± 1°C, pH 7.5 ± 0.5, O_2 6.5 ± 1 ppm. Two experimental diets consisting of 0 and 150 mg/Kg feed of PTS/PTSO in proportion 1:1; w/w were formulated for this purpose.

Results

The susceptibility of bacterial pathogens responsible for main diseases in *S. aurata* is shown in figure 1. All pathogenic strains proved to be sensitive to both compounds. PTSO exhibited a highly inhibitory effect ($\geq$ 20 mm) against all target strains from 10 mg/mL, whereas PTS had such effect from 25 mg/mL.

The fish fed with PTS/PTSO presented a higher probability of survival ($p<0.01$) compared with those fed the basal diet (Figure 1). The survival probability values in the CONTROL group were 66.7%, while, in the ALLLIUM group was 91.1%.

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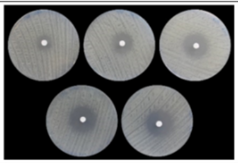
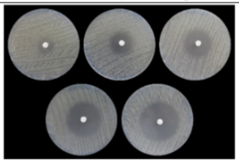
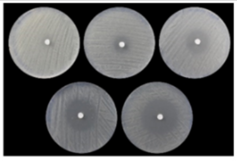
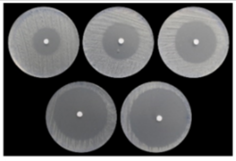
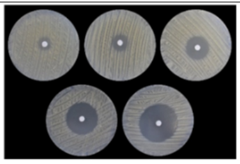
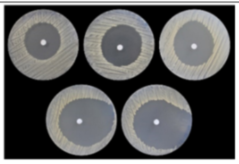
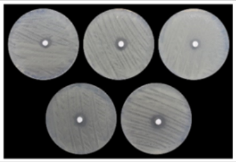
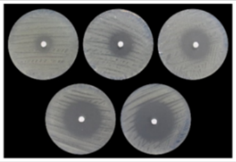
Microorganism	PTS (at 2.5, 5, 10, 25 and 50 mg/mL)	PTSO (at 2.5, 5, 10, 25 and 50 mg/mL)
P. anguilliseptica CECT 899		
T. maritimum CECT 4276		
P. damselae subsp. piscicida *DSMZ 22834*		
P. damselae subsp. damselae *CECT 5062*		

Figure 1. Results of the antibiosis test in solid media of PTS and PTSO at different concentrations against target strains.

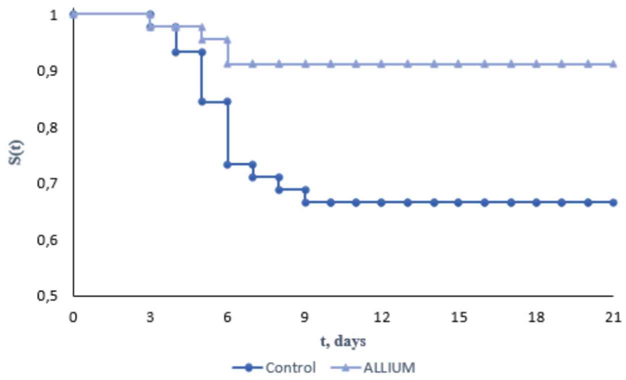


Figure 2. Kaplan Meier Survival curves for seabream (*S. aurata*) during 21 days of challenge test compared to control by Long-rank test at 95% confidence level.

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EVALUATION OF FERMENTED COMPOUNDS AS FISH FEED INGREDIENTS IN SEA BREAM AND SEA BASS

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Introduction

As marine aquaculture does not depend on fresh water sources and does not compete for space with terrestrial agriculture, it is considered to play a vital role in feeding the growing world population. However, a problem with modern finfish aquaculture is that it largely depends on fish meal coming from already overexploited natural fish populations as its main protein compound. Although considerable progress has been made in substituting animal protein for vegetable protein in fish feeds, further reductions are still required and there is continued research in finding relatively cheap, high quality and easily digestible protein.

The EU Horizon 2020-funded Innovation Action project SIMBA has the aim to create a better EU Agro-Aqua-Food system using microbiomes. One of the tasks of the WP dedicated to marine microbiomes is the optimisation of fish feed for more sustainable fish farming. This included the optimisation of fermentation mixes to produce high protein fermented vegetal products that can (in part) substitute less sustainable protein sources in fish food, such as fish meal. In this work we present the results of an evaluation of new feed mixes containing fermented compounds in the important Mediterranean finfish aquaculture species gilthead sea bream and sea bass.

Material and methods

Effects of dietary administration of rapeseed meal-seaweed fermented with lactic acid bacteria (EP199, 15% addition substituting 50% of fish meal) and soybean meal-seaweed fermented with lactic acid bacteria (EP299, 30% addition substituting 50% of fish meal) were evaluated on fish performance parameters, biometric and somatic indices, variation of gut microbiota profiles (analyses pending), water quality, main innate immune system indicators and final fillet quality in gilthead seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). A total of 720 gilt head bream and European seabass juveniles of 10.73 ± 0.02 and 9.13 ± 0.01 mean body weight respectively, were grouped into 6 treatment diets and fed for 12 weeks. Effects of the diets were compared to those of a standard commercial diet.

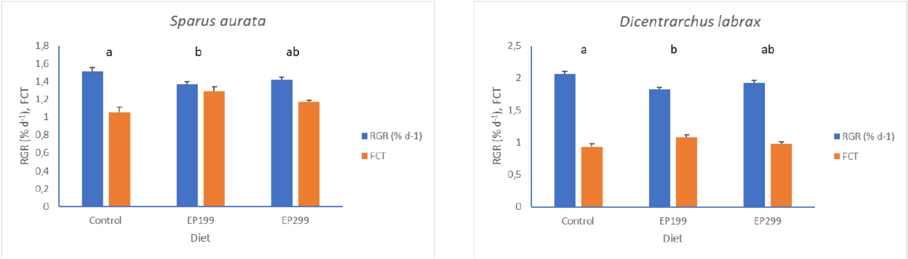

Fig. 1: Fish performance (growth rate and feed conversion rate) in two experimental diets containing fermented compounds (EP199 and EP299) to a standard control diet in *Sparus aurata* (left) and *Dicentrarchus labrax* (right).

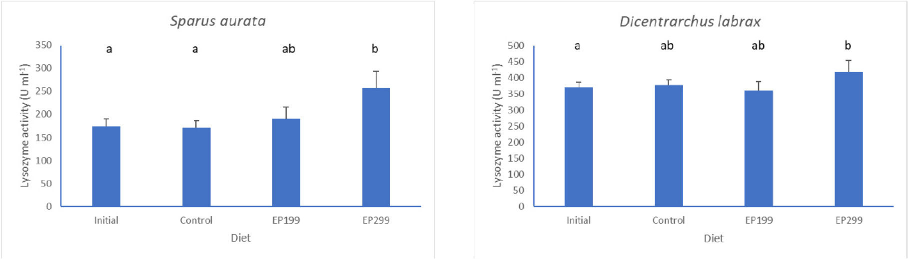

Fig. 2: Lysozyme activity in *Sparus aurata* (left) and *Dicentrarchus labrax* (right) fed two experimental diets containing fermented compounds compared to a standard control diet and initial values.

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Results and discussion

Fish performance as measured by growth rate and feed conversion was significantly lower (± 10%) for both fish species in the feed containing the EP199 compound than the control, whereas there was no significant difference for the EP299 feeds. The same pattern was observed for feed conversion, the best conversion was found for the control (Fig. 1).

Considering innate immune system indicators, the most obvious effects were found for lysozyme activity, especially in sea bream. Higher values were found for both EP199 and EP299 feeds, however the difference was only significant for EP299. For sea bass the differences were not significant (Fig. 2). With respect to fillet composition of the fish, preliminary analyses show that there was generally no significant difference in proximate composition nor in fatty acid profiles. However, it appears there is an effect on amino acid composition, especially in sea bass.

Current results indicate that in particular the compound EP299 shows promise as a sustainable alternative for fish meal protein, without causing negative effects on growth. In addition, there might be a positive immunomodulating effect, although this appears to be species specific. Further studies should be directed towards optimising the compound mixture and inclusion percentage of the compounds in the feeds.

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HUSBANDRY PROCEDURES AFFECT HEART RATE OF BROOD-STOCK OF GILTHEAD SEA BREAM

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Introduction

Captive fish are confronted with human-induced stressors deemed necessary for their rearing, such as husbandry procedures. For this study, we explored husbandry procedures in gilthead sea bream (*Sparus aurata)* brood-stock. Gilthead sea bream are commonly cultured fish in Southern Europe and are highly valued and consumed. Little research has been published regarding husbandry stressors for the brood-stock of this species, albeit their immeasurable value for producers.

In this project, our aim was to measure the welfare effects of daily husbandry procedures, such as feeding, netting attempts, and tank cleaning, as well as prophylactic measures such as an antiparasitic bath, in brood-stock gilthead sea bream. Our objective was to measure the stress caused by these husbandry procedures by monitoring their heart rate before, during, and after the procedures. To achieve this goal, we implanted internal bio-loggers that collected the heart rate and internal temperature of the fish automatically, without having to manipulate the subjects at the time of the physiological recording.

Materials and Methods

Subjects and Housing Conditions

We implanted bio-loggers in 18 adult sea bream distributed in six 3,000 L cylindrical tanks (2 m diameter x 1 m, water level 90 cm). The subjects were housed with seven more fish in the tanks (with and without enrichment), which made a total of 10 fish per tank (mean weight 791.3 g), and were housed in this conditions for five months.

Surgery Procedure

We followed the steps developed by Mignucci et al. (2021). The subjects were anesthetized with 2-phenoxyethanol (0.5‰, Sigma–Aldrich), and maintained anaesthetised during the procedure with a gill bath of 0.25‰ 2-phenoxyethanol. After skin disinfection with 5% povidone-iodine (Betadine), a 2 cm abdominal incision was performed along the ventral midline to insert the bio-logger (DST milli HRT, 13 mm × 39.5 mm, 12 g, Star-Oddi, Iceland, www.star-oddi.com) in the intraperitoneal space of the thoracic cavity, in close proximity to the pericardium. The bio-logger was attached to the ventral thorax by one stitch of non-absorbable monofilament nylon suture and another one of silk suture. The abdominal incision was closed with absorbable monofilament glyconate suture. Right after the surgery the fish was placed in their experimental tank and monitored until full recovery.

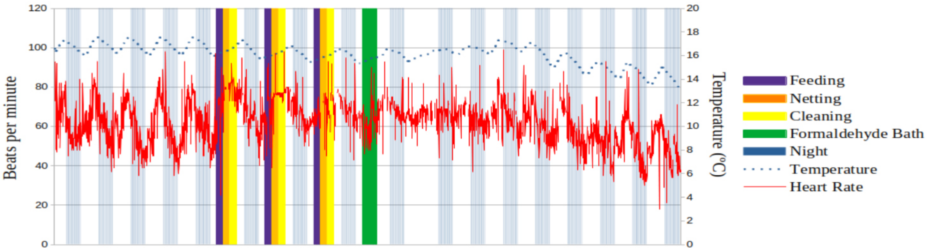

Fig. 1. Heart rate (red line) and temperature (blue dotted line) recordings of one adult gilthead sea bream over 17 days. Purple, orange, yellow and green columns represent the treatment events. Blue columns illustrate the night period.

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Treatments and Procedures
Treatment 1 - Feeding. We fed all the fish 0.5% of their body mass at 10:00 to measure the effect of feeding on their heart rate.

Treatment 2 - Routine net chasing simulation. We uncovered the tanks and moved a net (~2000 cm^2) on each tank at 11:00 for 5 minutes. The movement was from side to side and from the front to the back of the tank in a steady pace to simulate a catching event. We did not chase the fish with the nets and never caught any fish inside the net.

Treatment 3 - Routine cleaning simulation. At 12:00 we closed the air influx and cleaned the sides and bottom of the tank for 8 minutes with a broom (22 cm wide) while lowering the water 8 cm. We then waited 2 minutes for any dirt to sink and opened a purge system at the bottom of the tank for 10 seconds, which lowered the total water volume 10 cm more. We covered the tank and left the water reach its regular height.

Treatment 4 - Anti-parasite treatment. We lowered the water level up to 1/3 of the tank. We added 150 ppm formol (37% formaldehyde, <= 10% metanol, Sodacasa, Portugal) into the tank and left the fish undisturbed in the tank for one hour. After this time, the sides of the tank and any structure in them were cleaned with two brooms and we opened the drain and created a circular movement of the water by steering it with the two brooms, while we mixed it with clear water to eliminate most of the formaldehyde. The water level was 20 cm high, when we stopped the drainage and started filling up the tank. Two hours after the water had been filled up, we lowered the water level again to 1/3 of the tank, and filled it up with clean water. This step ensured any formaldehyde remaining in the tank would be diluted. The fish were not fed on the day of this treatment.

Treatments 1, 2 and 3, started five days after the surgeries and were repeated on three consecutive days. Treatment 4 was performed only once on the eighth day after surgeries. After the last treatment was performed, we continued monitoring the heart rate and temperature of the subjects for eight consecutive days.

Preliminary Results and Discussion

Data from one bio-logger (Fig. 1) showed a clear circadian rhythm of the heart rate with a decrease during the night and an increase during the day, a daily fluctuation between 40 and 80 bpm. The heart rate was maintained at high rates during the stressful events and consecutive hour, and it decreased overnight. However, consecutive days of stressors prevented the subject to reach its basal heart rate during the night. The final stressor, i.e., the prophylactic bath, maintained the heart rate of the individual above 60 bpm for the following day and night, and only got to its basal level on the sixth night after the treatment. Our preliminary results show that consecutive daily stressors and strong stressors such as prophylactic baths increase the heart rate of brood-stock gilthead sea bream, and one night of rest is not enough to reach their overnight basal levels. Gilthead sea bream might require several days to recover from strong stressors.

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METABOLIC RESPONSES TO SHORT STARVATION AND REFEEDING IN *Seriola dumerili*

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Introduction

Profitably maximizing production is one of aquaculture's priority objectives, focusing on optimizing feeding processes. In this regard, feed restriction has been artificially applied in aquaculture to induce compensatory growth without compromising fish growth. This phenomenon constitutes an effective strategy to control the fat content in the body of cultured animals and improve their feed quality, favoring an increase in feed intake and utilization (Argüello-Guevara *et al*., 2020). In addition, starvation can provide information on physiological processes without the interference of other factors derived from digestion and nutrient absorption processes (Barreto-Curiel *et al*., 2017). The greater amberjack (*Seriola dumerili*) is particularly interesting for the aquaculture sector due to its rapid growth rate, good performance in captivity, and high commercial value (García and Díaz, 1995); however, its cultivation in Spain is still incipient (APROMAR, 2021). Knowing how this species responds to some situations, such as starving, is an important challenge for aquaculture since it would help to prevent possible damage to fish health and, consequently, to optimize production. This work aimed to evaluate the metabolic strategies of *S. dumerili* to face a short period of starvation and subsequent refeeding.

Materials and methods

Greater amberjack juveniles were provided by a commercial source (Futuna Blue S.L., El Puerto de Santa María) and acclimated to the indoor experimental facilities at the *Servicio Central de Investigación en Cultivos Marinos* (SCI-CM, CASEM, University of Cadiz). After an initial acclimation period, 28 animals were used with an initial mean body mass of 54.6 ± 2.1 g and were fed a commercial diet for 4 weeks twice a day (CF). At the end of the continuous feeding period (94.3 ± 6.2 g), a biometric sampling was performed where biological samples were extracted from eight fish. The rest of the animals (n=20) were subjected to a starvation challenge for a week and subsequently, biological samples were extracted from eight of these specimens (St). The remaining animals (n=12) were re-fed for a week and, as with the previous two groups, biometric sampling was performed and biological samples were taken at the end of the experiment (RF). Different biometric, somatic, and feeding indexes were analyzed and some metabolic and endocrine parameters in plasma and liver.

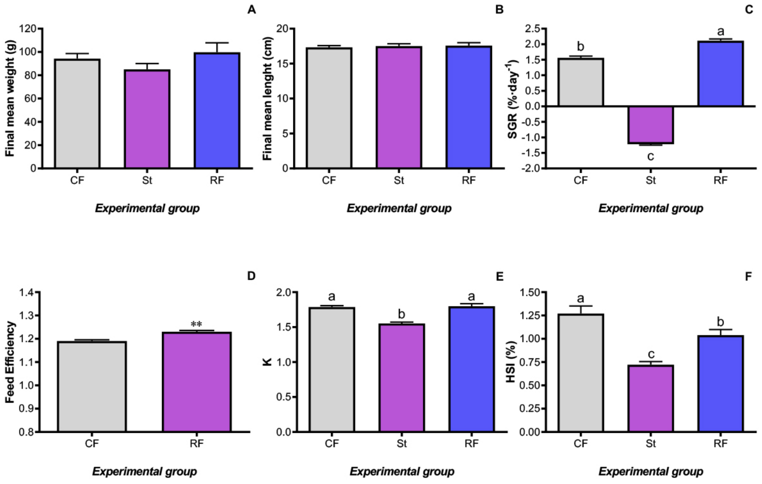

Fig. 1. A. Final mean weight; B. Final mean length; C. Specific growth rate (SGR); D. Feed efficiency; E. Condition factor (K); F. Hepatosomatic index (HSI) in *Seriola dumerili*. Results are expressed as the mean ± SEM for each group.

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Results and discussion

Both CF and RF experimental groups had positive and continuous growth throughout the study period. The final mean weight and mean length obtained showed no significant differences between experimental groups, although both results were higher in the RF group (Fig. 1A, B). The specific growth rate (SGR) was higher in the RF group concerning the CF and St groups (Fig. 1C), whereas a decrease in the SGR was observed in the latter. In terms of feed efficiency, significantly higher values were obtained in RF individuals, that consumed a higher amount of feed than CF individuals (Fig. 1D). This fact suggests that fish experienced a state of craving and increased appetite leading to increased feed consumption after refeeding, which results in a higher increase in body weight (Liu *et al*., 2018). The condition factor (K) also showed significant differences between groups, being significantly higher in the CF and RF groups (Fig. 1E). On the other hand, liver weight (HSI) was affected by the fasting period (Fig. 1F).

To survive situations of fasting or starvation, fish mobilize their energy reserves, which imposes metabolic adjustments that depend on the species, age, nutritional status, etc. (Navarro and Gutiérrez, 1995). Regarding plasma metabolites, no significant differences were found in glucose and lactate levels, although an increase was observed in the RF group. Triglyceride, cholesterol, and protein concentrations followed a similar trend, being significantly lower in the St group when compared with the CF and RF groups. At the hepatic level, a significant increase in glucose was observed in starved and refed animals, whereas hepatic glycogen showed no differences. Lactate and TAG levels were similar in the three groups, although with a tendency to increase in St and RF (data not shown). These observations can be also attributed to higher plasma cortisol levels in re-fed fish. Moderate increases in cortisol in the body stimulate the production of neuropeptide Y (NPY), which promotes feed intake and feed anticipatory activity (Bernier *et al*., 2004), which would support the results obtained with the growth performance indices and metabolic parameters. In conclusion, compensatory growth in *S. dumerili* is possible after 14-day starvation followed by refeeding without affecting fish welfare. The fasting period was compensated in part by an increased feed intake and feed efficiency. These results represent a promising alternative to improve the feeding schedule of this specie.

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EFFECT OF FUNCTIONAL ADDITIVES ON GROWTH PERFORMANCE AND WELFARE IN GILTHEAD SEA BREAM (*Sparus aurata*) JUVENILES FED WITH PLANT-BASED DIETS

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Introduction

The feed used in aquaculture is highly dependent on fishmeal (FM) and fish oil (FO) as the primary source of nutrients. The declining availability of these products, coupled with the rising costs of fishmeal (FAO, 2018), has prompted research into alternative sources of protein, and in particular into the use of plant-based proteins (Naylor *et al*., 2009). The use of healthy diets that include ingredients/additives with functional properties on the organism is a very promising option (Gatesoupe, 2005). In this context, nutraceuticals develop a fundamental role, improving numerous benefits for the health and physiological state of the animals, including the prevention or treatment of diseases (Burr *et al*., 2005). The aim of this work was to evaluate the potential benefits of two functional feeds for the gilthead seabream (*S. aurata*), which contain low inclusion of two microalgae-based products in vegetized diets, named as LB-ImmunoBoost (IB) and LB-LiverProtect (LP).

Materials and methods

Four diets were formulated at the Experimental feeds Service facilities (University of Almería, Spain). The control diet (C+) did not include any functional additives, with a nutritional composition based on fishmeal and fish oil. The second diet consisted of a high percentage of fishmeal and fish oil substitution (75%) by vegetal proteins and oils (C-). In addition, the C- diet was supplemented with 1% of LB-ImmunoBoost and with 1% of LB-LiverProtect, constituting the IB and LP experimental diets, respectively. The feeding trial was carried out at the facilities of the Aquaculture Technological Centre (CTAQUA, El Puerto de Santa María, Cádiz, Spain). After an initial acclimation period of 14 days, juveniles with 27-28 g initial mean body weight were randomly distributed in twelve 400 L tanks (n=30 fish per tank). Experimental diets were offered to satiety (*ad libitum*), two times per day, 5-6 days per week for 90 days. At the end of the experimental period, final sampling was carried out when biometric parameters were measured for all specimens and biological samples were collected. Different biometric, somatic, and feeding indexes were analyzed. Plasma cortisol levels were measured with a commercial Cortisol Enzyme Immunoassay Kit. In addition, a representative panel of genes related to stress and growth pathways was performed by semi-quantitative PCR with a CFX96 Real-Time System and expressed as relative gene expression (ΔΔCq Normalize Expression Gene Study) by using the Bio-Rad CFX Manager™ 3.1 software.

Results and discussion

Diets with high levels of substitution of animal ingredients by plant sources achieve good growth rates as long as the feeds are balanced in terms of protein, lipids, and carbohydrates (Perera *et al*., 2020). Even so, the results showed better growth performance and metabolic rates in fish fed with C+, although the compound IB partially reverts the detrimental effects produced by the plant protein-based diets.

Gene expression of some genes related to the hypothalamus-pituitary-interrenal (HPI) and growth axes was studied in fish. In the hypothalamus, the expression of *crh*, *crhbp* and *nr3c1* genes were significantly higher in the C+ and IB diets than in the C- and LP diets, with clear negative feedback produced by plasma cortisol (Fig. 1). In the pituitary, *gh* expression was low in the C+ diet while *pomca* expression was significantly higher in the group fed the LP diet. On the other hand, *pomcb* expression was higher in the control diet and significantly lower in animals fed a high substitution of plant-based ingredients (C-), concomitantly with a higher expression of *ghr-I*, *ghr-II* and *nr3c1* genes in animals fed the C- diet. No significant differences in *star* expression were detected in the head kidney, whereas results on *11B-hydroxylase* showed a significantly lower expression in the two diets supplemented with the nutraceutical compounds. The *nr3c1* expression was higher in animals on the IB diet and lower in those fed LP.

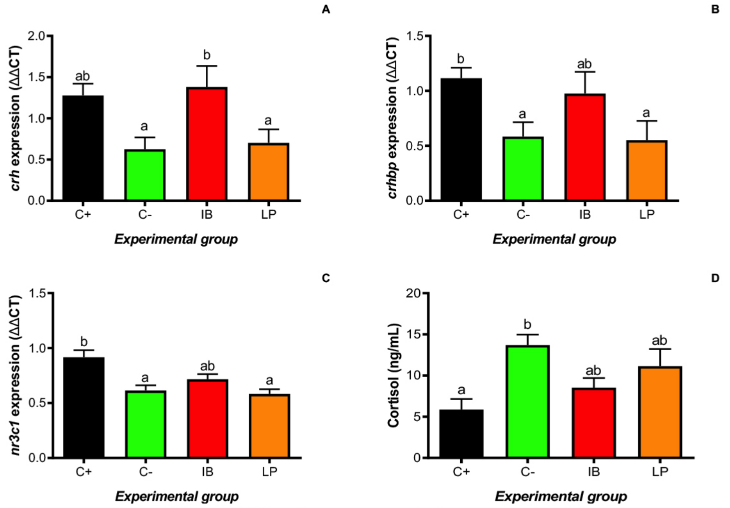

Fig. 1. Relative expression of three HPI axis genes and plasma cortisol levels concerning diet in *S. aurata*. A. *crh* gene; B. *crhbp* gene; C. and *nr3c1* gene; and D. cortisol. Results are expressed as the mean ± SEM of the triplicate tanks.

These results suggest that the expression of all aforementioned genes was significantly influenced by the dietary treatments, where the HPI axis is modulated at different central levels, orchestrating growth performance and welfare. Interestingly, the inclusion of the nutraceutical ImmunoBoost showed a decrease in stress levels and partially alleviated the negative effects of the substitution of raw materials in the diet, suggesting its potential use in aquafeeds.

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ASSESSING PHYSIOLOGICAL EFFECTS OF FEEDING AN INNOVATIVE DIET IN FARMED EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

Fish production from aquaculture has expanded greatly during the last decades, and aquaculture is recognized as a major food production industry (FAO, 2020). However, concerns about environmental issues, sustainability and animal welfare in aquaculture are increasing. About sustainability of aquaculture, major concern is related to use of fish meal and oil in fish feeds that are coming from wild caught fish (Naylor et al., 2021). In this study, we evaluate the effects of an innovative diet (with replacement by a new yeast-based protein ingredient) on the health and welfare indicators of European sea bass (*Dicentrarchus labrax*), a key species of the European marine aquaculture. In this work, we monitored level of stress molecular indicator (*HSP70*), physiological blood parameters of interest (e.g. haemoglobin, cortisol, glucose, lactate, lysozyme), as well as the growth performances of sea bass reared in sea cages fed the innovative diet. In addition, a sub-sample of fish has been implanted with accelerometer tag for continuous monitoring of acceleration, a proxy of energy expenditure (Carbonara et al., 2021), for about two months.

Materials and methods

European sea bass were monitored from May 2020 to July 2021. Feeding trial started on August of 2020. Six experimental cages were used (n=~2200 fish per cage) in the farm of AVRAMAR at Palairos (Greece). Fish were fed two feeds; a commercially available control feed which was offered in three cages and the experimental innovative- diet which was offered in the remaining three cages. Innovative diet was based on the control diet but with reduced fishmeal inclusion aiming to reduce FIFO (Fish in:Fish out) ratio. Throughout the experimental period fish were fed *ad libitum*, 2 – 4 times daily depending on their weight and the water temperature. Sea water parameters such as water temperature and dissolved oxygen were recorded daily. Over the trial, three samplings were carried out at three different times (T0: in May 2020 'control' before starting the feeding, T1: in April 2021 and T2 in July 2021). At the three sample times, growth measurements and blood and organs samples were carried out. At T1, n=5 fish per cage were tagged using accelerometer tags with 'tailbeat mode' algorithm, allowing to measure fish acceleration (m/s^2), which is a proxy of energy expenditure (Carbonara et al., 2021). At each sampling point, fish were gently caught from the rearing tanks or cages and bathed into anesthetic (clove oil) for 2-3 min before proceeding to blood sampling. The blood samples were taken from the caudal vein, and used to assess the basal levels of the following physiological indicators: cortisol, glucose, lactate, hematocrit, hemoglobin, red blood cell count (RBCC), noradrenaline, adrenaline and lysozyme. The quantification of these parameters was performed as described in Carbonara et al., (2019). Also, the levels of total proteins, prealbumine, albumin, alfa1, alfa2, beta1, beta2, Gamma and Immunoglobulin M in plasma were assessed according manufacturer instructions. Organs (spleen, liver, kidney, gills and brain) were also sampled to further quantify HSP70 though quantitative rRT-PCR.

Results and discussion

At the end of the trial, fish growth rates differed between treatments only in the final sampling; the average weight of fish fed the commercial diet was significantly higher compared to the average weight of fish fed the innovative diet ($p<0.05$). Similarly, SGR was found greater in fish fed the commercial diet than fish fed innovative one ($p<0.05$). Despite the significant greater feed consumption from the commercial group ($p<0.05$), the FCR, K-factor and the total mortalities showed no significant differences between feed treatments

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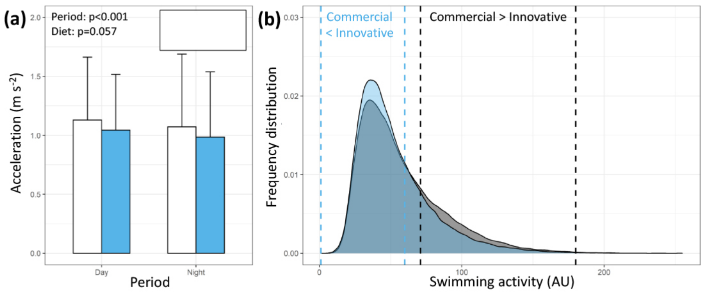

Fig. 1. (a) Acceleration (mean±SD; m/s^2) in fish fed commercial (white) or innovative diet (blue) during day and nightime. **(b)** Frequency distribution of the swimming activity values recorded in fish fed commercial (black) and innovative diet (blue).

Based on the physiological sensors data, sea bass displayed a diurnal pattern regarding acceleration; they were more active during daytime than nighttime. This was consistent with what has been observed in previous experiments where fish were more active during feeding at daytime (Carbonara et al., 2021). Only a tendency for significant difference between the two feeds (p=0.057) has been observed (i.e., fish fed innovative diet tended to display lower acceleration; Figure 1.a). When looking at the distribution of swimming activity values recorded by sensor, fish fed commercial diet displayed greater number of low activity values than fish fed innovative one, and oppositely fish fed commercial diet displayed lower number of low activity values than fish fed innovative one (Figure 1.b). This could be indicative of greater use of anaerobic metabolism for fish fed commercial diet.

Finally, regarding all the physiological parameters measured in plasma during the trial, only few were affected by the innovative diet. In more details, we found lower level of cortisol, glucose and hemoglobin in sea bass feed innovative diet than fish fed commercial one (p<0.05). Also, we measured greater level of total proteins in sea bass feed innovative diet than fish fed commercial one (p<0.05 for all). Some change in stress parameters (i.e. cortisol, glucose) has been measured punctually in one of different sampling times but levels were returned to similar level in other sampling time, suggesting that this change should be linked more to sampling time than diet.

Overall, this study provides a global assessment of sea bass physiology under innovative diet coping with sustainability challenges of European aquaculture. At the end of trial, the average weight difference between the two diet treatments was 30g, a weight that can be gained between 10-20 days depending of year period in the Mediterranean Sea. In addition, the health and welfare of sea bass was not significantly affected by this feeding. In conclusion, such innovative feed could be promising for the future of sustainability in the European aquaculture.

Acknowledgments

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EARLY INTRODUCTION OF MICRODIET IN SEABASS (D. LABRAX) LARVAE: EFFECTS OF TOTAL ROTIFER SUBSTITUTION AND ARTEMIA REDUCTION ON LARVAL DEVELOPMENT AND GROWTH PERFORMANCE

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Introduction

The global production of European Seabass, *Dicentrarchus labrax,* has increased constantly from close to 71,000 Metric Tonnes (MT) in 2000 to 236,000 MT in 2019.

The production of good quality live prey is a basic requirement for good fry production. Although hatcheries have achieved high standards of production, this type of activity could present some difficulties due to the variability of the nutritional quality of the product and possible production problems, which affects the survival and quality of the fish produced.

In order to increase flexibility in terms of production, the aquaculture industry is moving towards partial live food using specifically formulated diets in the larval rearing phase.

The present study, conducted on a commercial scale, investigated the effect of different feeding protocols in the regime of live prey replacement by introducing dry feed from the first days of exogenous feeding. The scope is to evaluate the effect of the dry feed in combination with different levels of live food reduction focusing on the period in which Artemia is fundamental for the growth of the seabass larvae.

Materials and methods

Seabass experiment

Hatched Seabass larvae, originating from the same pool of eggs, were stocked at the density of around 60 larvae.l^{-1} in 6,000l larval rearing tanks. Different feeding protocols were used to evaluate the best combination of dry feed and live feed from 0 to 56dph.

In all treatments, *Artemia* has been fed from 9dph onwards and early weaning was completed by 45dph.

A photoperiod of 14L/10D was used and the treatments were done in duplicate.

During the larval rearing period, from 7dph weekly biometrics were carried out to compare growth rates. Salinity stress tests were done to determine the stress resistance. At 56dph, growth, larval survival, produced biomass per tank, and deformity levels were evaluated.

Results

The Seabass larvae reared in a regime of reduced live food and use of dry diets, showed similar performance in terms of growth and stress resistance if compared with a standard feeding protocol.

Conclusions

This study shows that Seabass larvae can be reared obtaining a good survival rate and good fry quality when a balanced diet is used from the first days of exogenous feeding at the same time as a careful replacement of Artemia in the right moment of larval development.

The use of a high-quality diet is fundamental to obtain optimal growth and quality in the larval rearing of Seabass. Even in the absence of rotifers, the partial replacement of Artemia is an achievable goal, optimizing its use during the most critical phase of larval rearing.

DIETARY SUPPLEMENTATION WITH OSTEOGENIC EXTRACTS DURING THE PRE-ONGROWING PHASE IMPROVES SKELETAL QUALITY OF GILTHEAD SEABREAM AT THE ONGROWING PHASE

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Introduction

The high incidence of skeletal deformities in farmed gilthead seabream (*Sparus aurata*) is a major concern due to the negative impacts on fish market value and animal welfare[1,2]mainly in the hemal and caudal body regions. Four hundred and twenty-two hatchery-reared sea bream from Italy, France and Spain were checked for skeletal malformations and meristic counts. The same skeletal descriptors were examined in 72 wild-caught sea bream. Correspondence analysis (CA. Dietary supplementation of natural extracts rich in compounds with health-promoting effects on the skeleton offers a cost-effective strategy to reduce the incidence of deformities in farmed fish[3].

Materials and Methods

Experimental diets (Sparos Lda) were supplemented with ethanolic extracts prepared from the microalgae *Skeletonema costatum* (SKLT) and *Tetraselmis* sp. CTP4 (CTP4) and fed to gilthead seabream juveniles (~5 g; reared in triplicates in 250 L tanks at a density of 1.7 individual/L) until triplication of their weight (33 days). A first sampling (T1) for growth, hematological parameters, and skeletal quality was conducted on ~45 fish (~15g of individual weight) per condition. The remaining fish were fed with a commercial diet for an additional period of 6 months to evaluate if short-term dietary supplementation during the pre-ongrowing phase could yield an improved seabream quality in the ongrowing phase. A final sampling (T2) was conducted on 40 fish per diet and skeletal quality and shape differences were determined from radiographic images through morphometric analyses (**Figure 1**).

Results

Results from T1 revealed that fish fed with the CTP4 diet weighed more than fish from the control group ($p<0.05$); however, there were no significant differences in the total length (TL) at this time. Interestingly, results from T2 revealed that both the SKLT and CTP4 dietary groups produced longer fish than the control group (TL, $p<0.05$). Fish fed with the CTP4 diet displayed a lower incidence of severe anomalies at both T1 and T2. A Principal Component Analysis (PCA) applied to morphometric features identified on radiographs from T2 revealed that dietary conditions can explain the shape differences between groups (**Figure 2**). Furthermore, Thin Plate Spline warp analyses suggest that fish from the CTP4 condition are characterized by slender and elongated features.

Discussion

Our results indicate that dietary supplementation with ethanolic extracts of Skeletonema costatum and Tetraselmis sp. CTP4 applied during a brief pre-ongrowing window, is able to improve juvenile seabream growth and skeletal health with long-lasting beneficial effects. Furthermore, fish fed with the CTP4-supplemented diets display more elongated and slenderer morphometrical features compared to control fish. It is well known how fish external appearance influences the product consumer perception and therefore its profitability4. Our finding highlights that choices in feed formulations may represent a useful tool that farmers could use to intervene on fish shape and external appearance.

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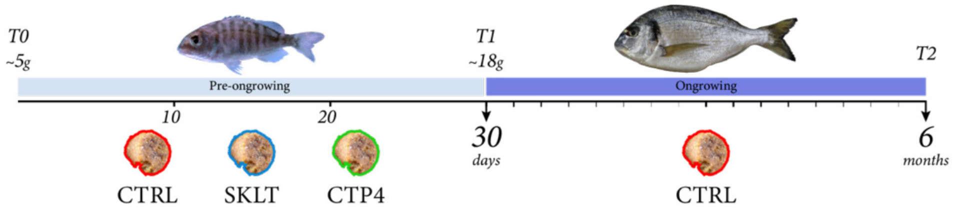

Figure 1. Schematic displaying the experiment timeline.

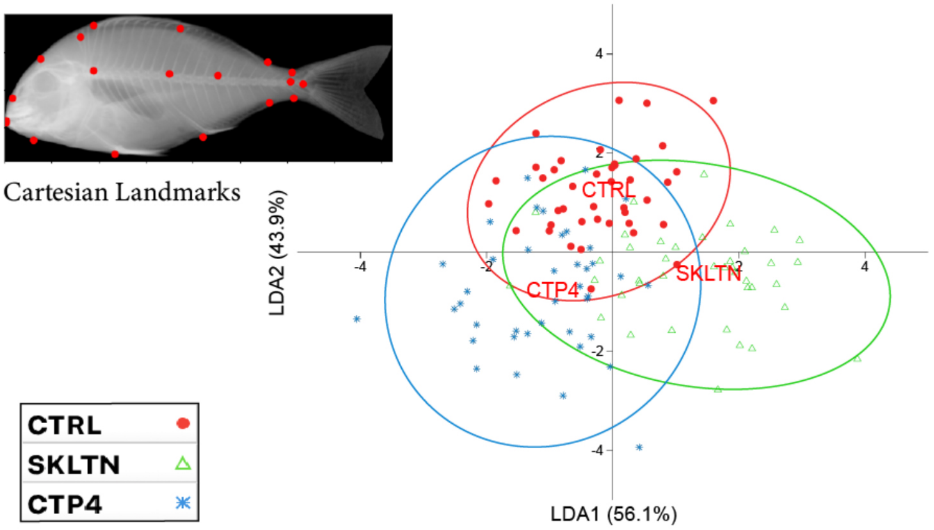

Figure 2. The PCA revealed significant differences in fish shape among all the three dietary treatment groups (Wilks' λ, p<0.00001). Pairwise Mahalanobis squared distances were significantly different between all the conditions (post hoc with Bonferroni corrections, p<0.00001).

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SAFER PROJECT - IMPROVING THE INNOVATION PERFORMANCE OF THE SEAFOOD SECTOR BY INCREASING TECHNOLOGY ADOPTION AND TRANSNATIONAL COOPERATION IN THE ATLANTIC AREA

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Introduction

The seafood sector is becoming more and more important in the present context of growing world population and consequent increase in food demand. Seafood not only represents the main source of proteins for millions of people in the world but also generates a lower carbon footprint than animal production in land. Atlantic countries have a strong stake in the growth of the seafood sector, both in terms of production and consumption. In 2017, United Kingdom, France and Spain were ranked in the top five of the main EU aquaculture producers. EU aquaculture is characterised by its high specialisation at regional and country level and is strongly influenced by geography and the natural habitat of the species, which makes the Atlantic Ocean a major asset for its coastal regions.

In order to continue to meet the growing demand for aquaculture products, while balancing with sustainability goals and the preservation of natural heritage, the seafood industry needs to respond to different challenges and opportunities requiring innovative solutions.

The SAFER Project was started in 2017 to respond to these challenges in the Atlantic area. SAFER aims to boost the adoption of technology and transnational cooperation and to improve the innovation performance of the seafood sector in the Atlantic regions and here are some of the results and perspectives of the project.

Methodology

SAFER (Smart Atlantic Seafood Clusters) is developed in three complementary actions:

1. A unique innovation services platform available online and designed for stakeholders from the seafood sector. It provides a catalogue of more than 80 services for effective organisation, governance, and innovation best practices in the Atlantic.
2. Seven living lab pilots in the Atlantic area developed to apply these tools in different companies involved at different steps of the seafood value chain and foster innovation adoption.

3. The production of recommendations for the Regional Innovation Smart Specialisation Strategies (RIS3), which will help the transfer of services to other businesses and local actors in the Atlantic.

Promoting innovation throughout the seafood value chain

Inspiring technology and collaboration within the seafood sector is at the heart of the SAFER project. The implementation of pilots is the most effective and tangible opportunity to illustrate the benefits of the innovation platform and its potential to be replicated by seafood actors in the future, across the Atlantic and beyond. The SAFER project jointly implemented 7 living lab pilots of innovative Industry 4.0 technologies, all based on a tailor-made approach of the 5 Atlantic regional nodes' needs and expectations.

The pilots implemented are a good example in terms of sustainability. They helped to reduce the environmental impact, i.e., the marine water treatment pilot minimises the marine water demand due to lower water exchange requirements with the surrounding media. They also helped to reduce the impact on the carbon footprint through a more effective management of stocks, logistics and production processes as it is the case of the Manufacturing Production Control Systems implemented in Ireland, France and Portugal.

Regional Smart Specialisations: an ocean of potentialities for research & innovation in the seafood sector

The SAFER pilots, in addition to concretely contribute to the transfer of innovation from one company to another, also offers an interesting source of information in the development of regional policies, in particular the so-called Regional Smart Specialisation Strategies (S3).

Smart Specialisation Strategies are a tool to support investments in key regional sectors with a competitive potential. Given the bottom-up approach of S3s, they implicitly support the creation of partnerships among different territorial actors of specific sector of specialisation, such as academia, business, and research centres for the development of a long-term sustainable growth.

The seafood sector features prominently in the Smart Specialisation Strategies of Atlantic Regions. The analysis of the S3s of Atlantic Regions showed that 15 Atlantic regions selected fisheries and aquaculture as a S3 in the period 2014-2020.

Among these regions, Cantabria Region (Spain) directly used SAFER as a mean to gather regional stakeholders of the seafood value chain and support the seafood chapter of its new regional S3 in 2021-2027.

It can be observed a general maintain of the seafood sector in the Atlantic regions' S3 for the new Multi-Annual Financial Framework (2021-2027), though not all the Regional Smart Specialisation Strategies have not been published yet. The regions share the view that the sector has economic, social, and environmental potential for regional growth given the increasing global demand for seafood products and high-quality healthy food.

In this context, SAFER will remain pertinent to support further guide the implementation of S3 related to the seafood sector.

Conclusion and perspectives

Seafood is a strategic blue economy sector for Atlantic regions and plays a vital role in the present context of increasing food demand and need for sustainable and resilient food systems. However, the sector must face several challenges to compete at the global scale while at the same time protecting the marine environment and avoiding additional pressures on already fragile ecosystems.

Hence, innovation is key to modernise this traditional sector and improve its sustainability, competitivity and resilience. The SAFER project, answering to these challenges and using the resources shared by a transnational network of different stakeholders, unlocked the innovative potential of the sector in Atlantic regions by testing and implementing living lab pilots which successfully helped mature established businesses to adopt technology and increase, in this way, final product quality, ensuring product traceability and reducing industrial costs. All these technologies promote the improvement of processes and products which comes at a crucial moment and paves the way for the sustainable transition of the Atlantic seafood sector.

SAFER 2.0 current and future actions

SAFER is exploring the concept of Industry 5.0 in the seafood sector. Industry 5.0 is a new concept which provides a vision of industry that aims beyond efficiency and productivity as the sole goals and reinforces the role and the contribution of industry to society.

It places the wellbeing of the worker at the centre of the production process and uses new technologies to provide prosperity beyond jobs and growth while respecting the production limits of the planet. This is of special relevance to the seafood sector where a sustainable management of sea resources is crucial.

THE EELS OF NORTH ADRIATIC LAGOONS AT DIFFERENT SILVERING STAGES: TOO YOUNG TO REPRODUCE?

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Introduction

The European eel (*Anguilla anguilla*, Linnaeus 1758) is a catadromous fish with significant cultural, scientific, and commercial value. The population has declined alarmingly over the past 30 years (Van den Thillart *et al.*,2009; Dekker *et al.*, 2018); this condition has led to the classifying of this species as "critically endangered" according to the International Union for Conservation of Nature (IUCN). One of the conservation measures taken for this species is artificial reproduction, which has yielded excellent results over the last 10 years (Mordenti *et al.*, 2013; Di Biase *et al.*,2016). This work aims to study North Adriatic populations of European eel and test artificial reproduction on animals considered residents (yellow eel), pre-migrants (i.e., not ready to migrate) and migrants (i.e., ready to migrate and consequently reproduce) by the Durif *et al.* (2005) classification.

Materials and methods

A total of 854 European eels were caught in the North Adriatic lagoons (n.7). All eels caught were subjected to the detection of morphometric parameters. The initial stage of eels relative to the silvering process (Silver Index-SI) was determined according to the classification system described by Durif *et al.* (2005). Gonads were collected from a sample of eels (10%) for human consumption from each lagoon and the gonadosomatic index (GSI) was calculated. Age was determined by examining scales in 50% of the eels caught. To compare reproductive potential, n.48 females (14 SI II residents, 21 SI III pre-migrant, and 21 SI IV/V migrants) underwent induced reproduction following the originally developed protocol by Mordenti *et al.* (2018). For each spawning event, the reproductive performance of the eels was analysed to identify differences in gonadal maturation, number of reproduced-spawned females (%) and number of eggs production (% BW) (Mordenti *et al.*, 2018).

Results

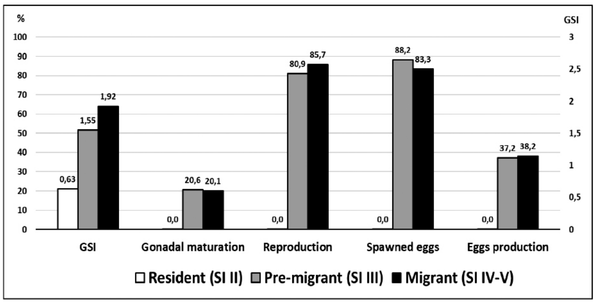

Fig.1 Main reproductive performance of the North Adriatic eels

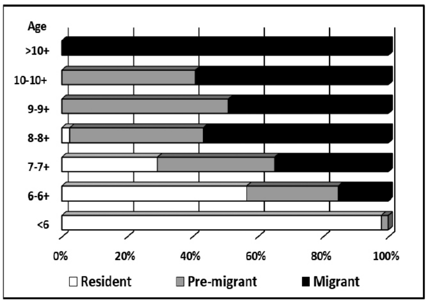

Fig. 2 Age classes of eels

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Discussion

This study aims to know about the North Adriatic eel population and to test the reproductive capacity of pre-migratory and migratory eels. The results are reported in Fig.1-2. Regarding artificial reproduction, both the hormone treatment and the number of spawned eggs (%BW) yielded similar values for both migratory and pre-migratory eels, while no positive response was observed in resident eels (Fig.1). The pre-migratory eels seem to have all the prerequisites to become fully-fledged eels ready for migration. Reinforcing the ability of these eels to reproduce is the fact that the pre-migratory eels (SI III) had almost the same gonadal maturation (GSI) as the migrating eels (SI IV-V) (Fig. 1). Furthermore, this could also be justified by the fact that we observed a certain overlap in the age of the pre-migrating eels with the migrating ones. Especially in the 8-10+ age range, pre-migrant and migrant eels are equally distributed (Fig. 2). Perhaps this can be explained by the fact that the transition from sedentary eel (SI II) to migrant eel (SI IV-V) is in our case a very rapid process that develops in a very short time (probably in just one growing season) in which the explosive weight gain is associated with a strong gameto-genetic intensity that is probably faster than complete metamorphosis (Gentile *et al*., 2022). The same study by Durif *et al*. (2005) argued that the silvering process is conditioned by various environmental factors that play a key role in this process. In fact, favorable growth conditions make the second metamorphosis of eels faster; the same favourable conditions encountered in the North Adriatic lagoons. Therefore, the functions developed by Durif *et al*. (2005) on Atlantic populations to distinguish silver eels cannot be fully adopted for Adriatic eels.

Conclusion

In general, this study increased knowledge North Adriatic population of European eels and demonstrated that eels considered pre-migrants have the same reproductive capabilities as migrants, and so pre-migrant eels can successfully contribute to sea release programs and artificial reproduction. With the contribution of the Life Programme of the European Union – Project LIFEEL (LIFE19-NAT/IT/000851).

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THE LOW TROPHIC GRID – NEW TECHNOLOGY TO ADAPT MONOCULTURE FISH SITES TO IMTA

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Introduction

Aquaculture continues to be a key pillar of future food production systems and there is an ambition within the sector for increased sustainability and circularity. Strategies such as the European Green Deal, World Ocean Initiative and Strategic Guidelines for a more Sustainable and Competitive EU Aquaculture, also promote innovation, integration and the adoption of a multi-sectoral approach, to maximize ecosystem services while providing social and economic benefits.

Integrated Multi Trophic Aquaculture (IMTA) is acknowledged as a promising solution for sustainable development of aquaculture. IMTA is shown to remove waste materials from fed species and lower the nutrient load in the water (FAO, 2018). Further development of IMTA and low-trophic aquaculture are two elements outlined in the EU strategy for sustainable aquaculture development which can play an important role in improving the environmental performance of EU aquaculture. Furthermore, changes in consumer attitudes has seen a parallel demand in low trophic products such as invertebrates and seaweeds (Barbier, 2019). Despite these development, the EU remains one of the world's largest importers of fisheries and aquaculture products, importing 70% of the EU consumption (STECF, 2021). There is a need to enable nutrient recapture and transition current production to a more circular model.

Technology, equipment and infrastructure were identified as 3 of 9 major issues facing IMTA by a recent report for the Blue Bio-economy (EUMOFA, 2020). The proposed technology adapted in this project, facilitates the natural ability shellfish and seaweed provide to recycle the nutrients or waste that are present in and around fish farms. This helps improve the environmental performance of aquaculture production sites, maximises the use of space and the greater diversity of species provides extra economic benefits.

Methodology

The Marine Institute operates a fully licensed pilot scale multi species research site on the West Coast of Ireland. Infrastructure on site consists of a traditional 6 x 50m circumference pen grid for fish culture, approximately 0.25 the size of a commercial pen. To increase diversity and quantity of lower trophic species, a significant upgrade to the mooring systems was made to better integrate the extractive species in a way that considered cost, visual impact, environmental impact, benthic impact and capital expenditure.

Situated downstream of the modelled nutrient dispersion from fish waste at the pens; the new Low Trophic Grid (LTG) is designed to create a pathway for current monoculture facilities to 'add' productivity to their sites. The grid model aims to maximize available space and minimize environmental impacts. The grid is comprised of two 60 x 35m submerged rectangular grid rings, secured to the existing mooring ring of the fish pens, at a depth of 3m. This expands the site footprint with capacity for an additional 780m of longlines. The design allows the operator to adjust orientation, depths, and spacing of culture lines using multiple attachment points to facilitate a range of low trophic species. This new LTG was moored as an annex to the existing grid structure for fish culture in September 2021 and was fully stocked in October 2021.

Results

The novel development of the LTG, adds to the sustainable research infrastructure at the site enhancing research and production methods/technologies. By creating this new structure there is increased habitat and biodiversity at the site for low trophic species, and a reduction in the environmental nutrient load, delivering a healthier production system.

As the grid structure is submerged there is less visual impact, suspended culture limits interaction with the benthic environment and by utilising the main grid for fish culture, the co-location maximises uptake of nutrients released from fish rearing activities and reduces the number of anchor points by 50% when compared to traditional longlines. Expansion of cultured species to 10, primarily extractive species across lower trophic levels such as primary consumers and macro algae production. First point harvest 2022, included 4 tonnes macroalgae and 2000 shellfish and other invertebrates. The increase in production from the site is discussed using data on biomass accumulation and crop yields to provide expected nutrient uptake rates, compositional make up and value of additional products to the site.

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Acknowledgments

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GUT MICROBIOME COMPOSITION OF TURBOT *(Scophthalmus maximus)* POST-LARVAE FED MICRODIETS WITH VARYING PROTEIN SOURCES

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Introduction

Turbot (*Scophthalmus maximus*) is an economically valuable flatfish species of great relevance for marine aquaculture mainly in Southern Europe. However, aquaculture production of this species is still hampered by high and variable survival rates during the early development stages at hatcheries, due in part to unknown larval nutritional requirements and detrimental microbiological conditions in the rearing system (e.g. the presence of pathogens). A number of studies has demonstrated that nutrition plays a key role in the modulation of fish microbial communities and consequently on their susceptibility to pathogenic infection (see the review of Ringo et al., 2016). Therefore, optimization of hatchery's protocols, through the development of premium microdiets that fulfil larvae and post-larvae nutritional requirements and maximize their resistance to pathogens, is essential to improve larvae and juvenile quantity and quality. In this context, the present study aimed to evaluate the microbiota responses in the gastrointestinal tracts (GIT) of turbot post-larvae fed microdiets with different combinations of marine and vegetable protein sources.

Material and Methods

Turbot post-larvae with 31 days after hatching (DAH) (initial body weight, IBW = 0.10g ±0.01) originated from FLATLANTIC hatchery (Praia de Mira, Portugal) were randomly assigned to 12 flat-bottom tanks in a closed water recirculating system (initial density of 1900 larvae/tank). Three isoproteic diets (62% dry matter)- a basal commercial-like diet, used as control diet (CTRL), and 2 experimental diets (D1 e D2) including different combinations of marine and vegetable protein sources (squid meal, krill meal, hydrolised fish proteins, wheat gluten, pea protein, fish meal) - were randomly assigned to those tanks. Dietary treatments were tested in 4 replicates. From 31-33 DAH turbot post-larvae were co-fed with enriched Artemia and one of the 3 microdiets. After 33 DAH until 66 DAH post-larvae were fed in intervals of 45min from 9h00 to 22h00 (automatic feeders) with dry microdiets exclusively. System water parameters were measured daily with commercial probes and adjusted to maintain temperature at 19 °C, oxygen saturation level above 90% and salinity at 20 g. L^{-1}. Fish were sampled at 31 (n=36 individuals/treatment) and 66 DAH (n=120 individuals/treatment) to determine length, weight, relative growth rate (RGR) and feed conversion rate (FCR). Survival rate in each treatment was determined at 66DAH. Moreover, post-larvae entire GIT (n = 4) were sampled at 43 and 54 DAH and pooled (one sample per replicate tank) for the assessment bacterial community analysis (high-throughput sequencing of the 16S rRNA gene).

Results

Although there were no differences in RGR, feed utilization (FCR) and survival amongst dietary treatments, post-larvae fed D1 diet presented lower weight when compared to CTRL group (Table 1). The PCO analysis of ASV composition showed that diet did not have a significant effect on the bacterial communities in the GIT of turbot post-larvae (PERMANOVA: F=1.024; P=0.368; Figure 1), yet age (i.e., DAH) was a significant predictor of the bacterial composition of fish (F=0.086; P=0.001). Bacterial composition had no clear dominance by any bacterial order and was mostly composed by proteobacterial ASVs (>49% of reads). Moreover, a taxon-specific statistical analysis revealed differential abundances among key bacterial groups, but no clear ordination base on diet was found when analyzing the fifty most abundant ASVs.

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Table 1. Growth performance indicators of turbot post-larvae throughout the experimental period fed the different diets (CTRL, D1, and D2).

		Diets			1-way Anova
		CTRL	D1	D2	*p-value*
	Age				
Weight (g)	66DAH	5.32±1.09^{b}	4.90±1.36^{a}	5.25±1.22^{ab}	0.024
Lenght (cm)	66DAH	6.59±0.51	6.64±0.68	6.73±0.58	0.258
RGR (% day^{-1})	31-66 DAH	12.1±0.44	11.7±0.78	12.1±0.48	0.560
FCR	31-66 DAH	0.52±0.08	0.60±0.10	0.56±0.07	0.435
Survival (%)	31-66 DAH	95.6±1.6	95.6±1.9	95.9±1.1	0.952

Values are expressed as mean ± SD. Different subscription letters (a, b) indicate differences between larvae from different dietary treatments at the same age ($p < 0.05$). Absence of letters indicates no statistical differences ($p > 0.05$).

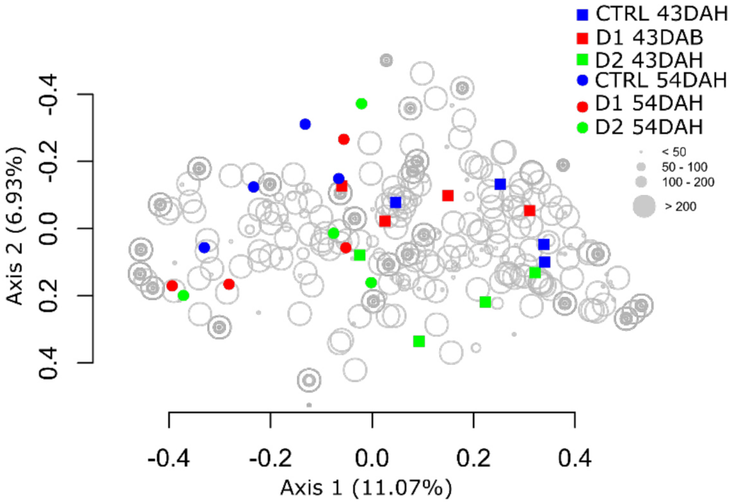

Figure 1 - First two axes of principal coordinates analysis (PCO) of ASV composition. Colored symbols are samples. Grey outlined symbols are weighted averages scores for ASV (size is proportional to abundance). Samples are control (CTRL) and experimental (D1 and D2) diets at 43 and 54 DAH.

Conclusion

Overall, the results showed that different microdiets tested had no major effects on the bacterial community in the GIT of turbot post-larvae. However, the zootechnical performance of turbot post-larvae fed with different diets showed that experimental diet D2 offers a viable alternative to commercially available microfeeds (CTRL diet) for turbot post-larvae.

Acknowledgements

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THE FATE OF DIETARY MICROPLASTICS: A MULTIDISCIPLINARY LABORATORY APPROACH TO EVALUATE LOCALIZATION AND PHYSIOLOGICAL RESPONSES OF ZEBRAFISH (*Danio rerio*) LARVAE

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Introduction

Nowadays plastic pollution is a worldwide problem and concerns all type of aquatic environment. Particularly, the presence of microplastics (size < 5 mm; MPs) has been found in the oceans constituting a source of pollution for aquatic organisms. Several studies have found the presence of MPs in marine animals, from the lowest trophic level to the top of the food chain. The presence of microplastics was also detected in sea-farmed aquatic species that showed similar rates of accumulation of MPs as those evidenced by wild specimens. More recently, the problem of MPs contamination in farmed fish has been extended also for land aquaculture. Fish meal products used in aquafeeds are made from fish-processing by-products or from low-value fish from wild fisheries. If recycling of by-products as feed ingredients contribute to support the sustainability livestock production their safety must be guaranteed not only for microbiological and chemical contaminants but even for microplastic. Furthermore, it has been shown that the concentration of MPs in fish meal is higher than that found in the raw materials from which it is derived. The reason lies in the fact that the processing procedures and packaging (polyethylene is one of the most widely used materials to produce "storage bags" for fishmeal) of the ingredients contribute significantly to the contamination of the feed. MPs contamination can have negative effects on both wild and farming fish production during different life-cycle stage. Many of these effects have been found primarily in the larval stages during which fish can exchange zooplankton for MPs resulting in gastrointestinal tract obstruction, a reduction in predatory activity caused by an apparent feeling of satiety, reduced growth and swimming capacity, and induction of inflammatory responses in the intestinal tract and other tissues because of translocation processes. However, despite the large number of publications on the presence of MPs in fish, little is known about their translocation after ingestion from the gastrointestinal tract to other target organs such as liver and muscle. The present study aims to assess the effects of a feed-induced MPs contamination during fish larval development, giving particular emphasis to growth and welfare, by monitoring the gut health status and the stress and immune response, and to the MPs fate to other target organs in relation to their size. In light of this, zebrafish (*Danio rerio*) represents an excellent experimental model being a fish widely used in nutrition and toxicology studies, with a short life cycle that allows to easily study its different phases under controlled conditions. Zebrafish has been widely used to study the effects of MPs exposure and the transparency of its embryos and larvae provides a significant experimental advantage for studying the tissue localization of fluorescent MPs.

Materials and Methods

A control diet containing fish meal as major protein source was prepared according to a commercially available standard diet for zebrafish (Zebrafeed, Sparos ltd, Portugal). Four experimental diets were prepared by adding to the basal diets two different sized fluorescent MPs (Polyethylene, 40-47 µm and an amino formaldehyde polymer, 1-5 µm), both from Cospherics (Santa Barbara, USA) at two different concentrations (0.05 g/kg and 0.5 g/kg). Zebrafish were fed the experimental diets (daily dose corresponding to the 3% of the body weight) from 5 to 21 days post fertilization. At the end of the trial, survival rate and specific growth rate were measured. To detect the presence of the fluorescent MPs ingested by the larvae and their possible translocation from the gastrointestinal tract to other organs, the whole specimens were analysed through Nikon A1R confocal microscope. For the quantification of the MPs accumulated by fish, larvae were chemically digested, and the product of digestion was filtered. Filters were then analysed through fluorescent microscopy to detect and count the number of MPs per larva. Histological analyses were performed to assess a possible damage to intestine absorptive epithelium induced by the MPs while molecular analyses were performed to analyse the relative expression of genes involved in immune (*il1b*, *tnfa*, and *il10*) and stress response (*nr3c1* and *hsp70.1*), appetite regulation (*ghrl*, *npy*, and *lepa*) and oxidative stress response (*SOD* and *CAT*).

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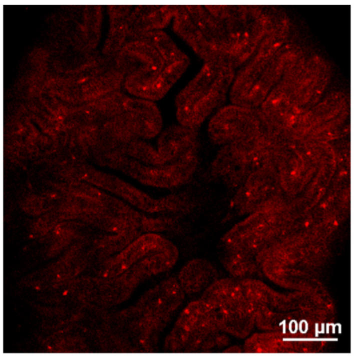

Fig. a: MPs detected by confocal microscopy in the intestinal villi of a zebrafish larvae fed a diet including the smaller MPs polymer at 0.05 g/kg. The size of the MPs beads (bright red) is of 1-5 µm.

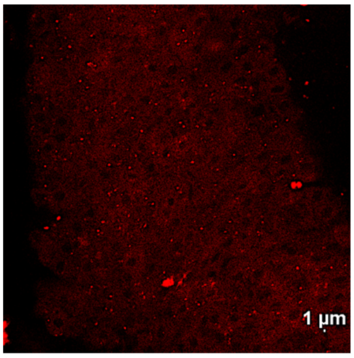

Fig. b: MPs detected by confocal microscopy in the liver of a Zebrafish larvae fed a diet including the smaller MPs polymer at 0.05 g/kg. The size of the MPs beads (bright red) is of 1 µm.

Results and Discussion

After the trial no differences in survival rate and growth were detected among the experimental groups. No MPs were detected in the control group, while their presence was observed in all the other experimental groups in a dose dependent way. Confocal microscopy evidenced that only 1-5 micron sized MPs were able to enter the intestinal epithelium (Fig. a) and only the 1 micron ones were found in the hepatic parenchyma (Fig. b) evidencing the presence of selective biological barriers.

The confocal microscopy results were coupled to the histological analyses on the gastrointestinal tract. Finally, fish fed with experimental diets showed a different expression of genes involved in stress, immune and oxidative stress response, and appetite regulation.

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THE EFFECTS OF FIVE METHIONINE FORMS ON THE GROWTH PERFORMANCE AND HEPATIC METABOLISM OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Methionine (Met) is an essential amino acid for the fish growth and it is notably under-represented in compound feeds based on plant protein sources, such as soybean meal. In order to meet fish nutritional requirement for Met, the strategy is to supplement their diet with synthetic (crystalline) forms of Met (Rolland et al., 2016; Yuan et al., 2011). However, the high leaching in the water and high intestinal absorption rates represent the main limits of the plant-based diets supplemented with crystalline amino acids. To overcome these limits, several technological processes have been developed, such as coating, encapsulation, microencapsulation, or polymerization (Guo et al.; 2020; Xiao et al., 2017). Currently, the Met sources used to supplement aquafeeds include L-Met and its derivative forms, such as DL-Met (the racemic mixture of D- and L-isomer of Met), and Methionine Hydroxy Analogue (MHA). The main MHAs are 2-hydroxy-4-methylthiobutanoic acid (HMTBa) and its calcium salt, HMTBa-Ca, both precursors of L-Met. DL-methionyl-DL-methionine (Met-Met) is a new dipeptide formed by dehydration and condensation of two DL-Met molecules. Moreover, the coated Met (Co-Met) has been used as exogenous feed supplements.

Accordingly, the aim of the present work was to evaluate the effects of different exogenous Met forms added to the rainbow trout diet on fish growth performance, hepatic SAM/SAH ratio levels, and mRNA copies of three genes: *SAHH* (S-adenosylhomocysteine hydrolase), *CBS* (cystathionine-beta-synthase), and *BHMT* (betaine-homocysteine methyltransferase) involved in the Met metabolism.

Materials and Methods

A two-month feeding trial was conducted with rainbow trout (*Oncorhynchus mykiss*) fingerlings (3.40 ± 0.40 g). Fish were divided into seven experimental groups with three tanks/group. The feeding protocol consisted in using two control diets, the first one being a FM-based diet (CTRL+) and the other one a vegetable meal-based with Met deficiency (CTRL-). To prepare the five experimental diets, 5 g of each Met form (Met-Met; L-Met; HMTBa; DL-Met, and Co DL-Met) was firstly mixed with fish oil and then added to the CTRL- diet. At the end of the feeding trial, the growth performance indices were calculated. Then, 3 fish per tank (9 fish per diet) were sampled for the whole-body composition (WBC), whereas 6 fish per diet were sampled for liver tissue analysis, which consisted in gene expression analyses of three genes: *SAHH, CBS*, *BHMT,* as well as the S-Adenosylmethionine (SAM) and S-Adenosylhomocysteine (SAH) quantification in HPLC (High Performance Liquid Chromatography).

Results and Discussion

Fish readily accepted the experimental diets and at the end of the feeding trial, they tripled their weight. However, no significant differences were found for the growth performance parameters between the different dietary groups. In a similar way, the performance indexes of red drum fed with MHA and DL-Met supplemented diets resulted not significantly different from fish fed with L-Met supplemented diets (Goff et al., 2004).

All the WBC parameters were affected by dietary treatments. In particular, Met-Met and L-Met promoted a significant body lipid reduction, whereas the protein retention was significantly increased in fish fed with HMTBa and Co DL-Met. These latter also affected the Met metabolism increasing the mRNA copies of *CBS* gene in the liver tissue, promoting an enhancement of the Met transulfur pathway. In agreement with our results, Zuo et al. (2019) found that HMTBa increased the transcripts level of *CBS* in hepatic cells from porcine. On the other hand, L-Met increased the expression of the *BHMT* gene, which is known to be involved in the Met re-methylation pathway, thus contributing to maintain the intracellular Met pool. Moreover, the Met free form, L-Met, increased the hepatic SAM and SAH concentrations, thus suggesting a higher Met bioavailability. Similar metabolic response was found in Atlantic salmon liver, too, in which Met intake affected hepatic SAM level (Espe et al., 2008).

With regard to *SAHH* gene expression, no significant differences were found between fish fed different diets, thus suggesting that the tested Met sources did not influence the methylation ratio (SAM/SAH).

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Conclusion

The present study showed an optimal dietary intake of the different exogenous Met sources with similar promoting effects on fish growth, and hepatic Met metabolism. Nevertheless, the mechanisms underlying these effects warrants further investigation.

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FROM FARM TO WHERE?
THE OBSTACLES OF SEAWEED-BASED SUPPLY CHAINS

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Introduction

Seaweed farming is a fast-growing aquaculture sector and is considered a more sustainable way of farming since does not require land, freshwater, fertilisers or pesticides (Baghel et al., 2020). It has been largely investigated for its potential in carbon sequestration and wide applications in food and feed industries, fertilizers and biostimulants, along with cosmetics, pharmaceuticals, bioplastics and biofuels (Collins et al., 2022)life cycle costing (LCC. Moreover, the prospect development of new supply chains based on cultivated seaweed could strengthen the path towards a circular blue bioeconomy, with food or bioremediation applications as well as promoting alternative revenue sources or employment opportunities to marine communities (European Commission, 2020). However, macroalgae cultivation is still in the early stages of development in the European context and several uncertainties underline the deployment and management of new supply chains based on macroalgae biomass (Araújo et al., 2021). Hence, the aim of this study was to enhance understanding of marine macroalgae cultivation from a management perspective towards responsible sourcing and new supply chains.

Material and methods

The study was based on a qualitative case study of emerging seawed cultivation sites in Ireland. Empirical case studies enable a profound investigation of a phenomenon of interest given a particular context (Rashid et al., 2019) and therefore particularly relevant to expanding understanding of the exponential interests involving marine macroalgae cultivation with wide different uses in the European context.

Results

The findings indicate several barriers to the expansion and deployment of seaweed businesses and supply chains, particularly in what relates to licensing, seed supply, processing methods and capacity as well as distribution. Moreover, seaweed supply chains from aquaculture are highly connected to institutional levels for planning and support involving policy, financing and regulations. Cooperation between seaweed farmers and research institutions suggests opportunities for development and innovation whereas cooperation between actors in the marine sector indicates a potential increase in bargaining power to overcome barriers and for business entrance or diversification.

Conclusion

Based on the study case in Ireland, multiple opportunities arise from the cultivation of marine macroalgae given the potential socio-environmental benefits seaweed can generate. However, multiple efforts are still needed, which from a grower's perspective involve trading potentials and commercialisation, government support of the aquaculture sector as well as technological development and transfer.

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MICROBIOME NETWORK ANALYSIS IN SKIN AND GILLS OF SPARUS AURATA FED WITH NANNOCLOROPSIS GADITANA MICROALGAE

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Introduction

One of the major current challenges of aquaculture industry involves the development of new commercial diets with the aim of reducing the unsustainable consumption of fishmeal while improving fish nutrition. In this context, *Nannochloropsis gaditana* is a microalgae rich in beneficial lipids, antioxidant compounds and amino acids, which has demonstrated improvements in growth and product quality in juvenile *Sparus aurata* (Ayala et al. 2020). In that sense, external barriers such as skin and gills can be used to measure how diets can affect and modulate the animal's metabolism. Both tissues are covered by a mucus secretion that includes a high number of microorganisms and molecular components of the immune system. The aim of the work is to observe what changes occur at the microbiota level in the skin and gills of commercial-sized *S. aurata* fed with a diet supplemented with raw and hydrolysed *N. gaditana* microalgae for 90 days.

Material and methods

N. gaditana biomass was produced in closed tubular photobioreactors at the Estación Experimental de las Palmerillas de la Fundación Cajamar following the standard procedure. Fresh *N. gaditana* was hydrolysed using commercial enzymes with cellulase activity (Vizcozyme®). The experimental feed was formulated by the Experimental Feed Service of the University of Almeria. The feeding trial was carried out at the facilities of the Central Service for Marine Culture Research of the University of Cadiz, where juvenile *Sparus aurata* weighing 10-15g were divided into 400L tanks and fed for 90 days with the study diets: a commercial diet (Control), a diet supplemented with hydrolysate and crude extract of *N.gaditana* at 5% (Hydrolysate and Crude, respectively). After 90 days, fish were anaesthetised and dissected for further analysis.

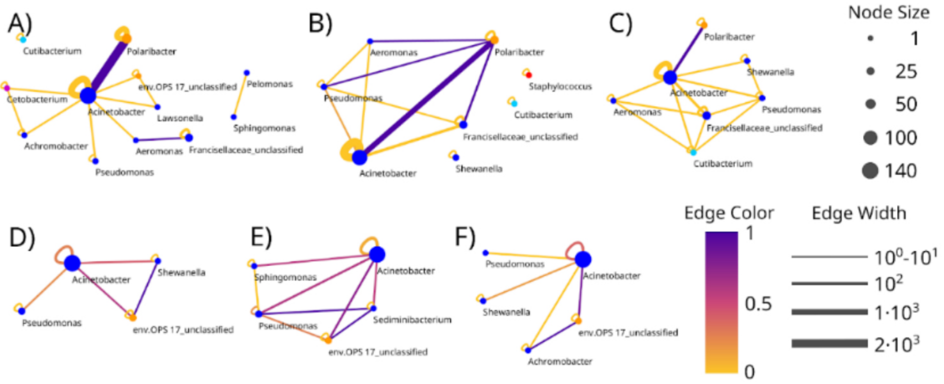

Figure 1. Genera involved in co-occurrence networks. Control diet (A), raw diet (B) and hydrolyzed diet (C) samples of gills. Control diet (D), raw diet (E) and hydrolyzed diet (F) samples of skin. The colour of the interactions is marked by the proportion of negative interactions.

(Continued on next page)

Skin and gill DNA was extracted according to Martinez et al., (1998). The 16S rRNA gene of the samples was sequenced using the Illumina® MiSeq platform (Illumina, San Diego, CA, USA) by pairwise sequencing (2 × 300 bp) at the Ultrasequencing Service of the Bioinnovation Centre of the University of Malaga (Malaga, Spain). Data processing was performed bioinformatically using a workflow based on the DADA2 library of R, using the SILVA 138 database with 99% clustering. Results were considered significant between Shannon index if t-student's $p<0.05$; while taxonomic comparison was performed using the R package DESeq2 ($p<0.05$). Co-occurrence networks were inferred from ASV abundances, based on Spearman correlation with the Hmisc library in R. Only Spearman correlations $p<0.01$ and with $\rho>|0.60|$ were considered statistically significant (Barberán, 2012) and visualised in Cytoscape 3.9.1. Only the ASVs present in more than half of the samples for each diet and tissue were considered in this network analysis.

Results and discussion

1416 and 694 ASVs in gills and skin respectively were preserved in the taxonomic analysis after filtering. The predominant *phylum* in gills was Proteobacteria (~50%) in the control and hydrolyzed groups and reached 70.58% in the raw diet. The Bacteroidota *phylum* was the most represented and Firmicutes and Actinobacteria were close to 3% in all treatments in this tissue. However, in all three different treatments, the phyla abundance was similar in skin samples, ~90% of which corresponded to Proteobacteria. Bateroidota (5.5%–5.9%), Firmicutes (1.8%–2%) and Actinobacteria (<1%) constitute the other phyla. Significant differences between treatments for each tissue were calculated. In gills, 13 significantly higher ASVs were obtained in the control (such as *Achromobacter*, *Acidobacter*) versus 4 whose abundance was higher in the hydrolyzed diet. The number of ASVs that differed significantly between the gill microbiota in the control vs. raw group amounted to 70, most of them being ASVs corresponding to the genus *Shewanella* (43) higher in the raw diet. Nevertheless, in the skin samples, Control group showed a significant increase of abundance related to *Acinetobacter*, *Achromobacter*, *Pseudomonas*, *Shewanella*, *Vibrio* and *Sphingomonas* among others. In the hydrolyzed group, the most significant abundance was associated with the genus *Pseudomonas*, *Vibrio*, *Pseudoalteromonas*, *Ralstonia* o *Cutibacterium*. In the case of the skin raw samples, there were ASVs corresponding to the genus *Acinetobacter*, *Streptococcus* or *Pseudoalteromonas* that were significantly different respect of control.

Taking the ASV abundance matrix for each diet and tissue, 6 co-occurrence networks were constructed. In all treatments in gills, *Acinetobacter* was a central genus in the network, and exhibited a negative correlation with *Polaribacter*. Besides, in the raw group, *Polaribacter* also showed negative correlation with *Aeromonas, Pseudomonas* and *Francisellacea*. In skin, *Acinetobacter* was involved in a major proportion of co-exclusion relations interacting with *Acinetobacter, Sphingomonas, Pseudomonas, Sediminibacterium* and env.OPS17 (Figure 1). This work shows that *Acinetobacter* has a key role in the balance of mucosa microbiota and was in co-exclusion with *Polaribacter*

Acknowledgments

This work was funded by research projects for young researchers, CEIMAR 2019 (Evaluation of hydrolysates of *Nannochloropsis gaditana* for use in high value-added finishing feed for farmed gilthead sea bream (*Sparus aurata*) (NAN2BREAM)).

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EVALUATION OF A NEUROSENSORIAL FEED ADDITIVE ON *Litopeneaus vannamei* SHRIMP STRESS REDUCTION

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Introduction

The marine shrimp aquaculture industry, in particular the whiteleg *shrimp Litopeneaus vannamei*, has experienced exponential technological development, with different production systems worldwide. Shrimp farming is made up of several stages and transitions that represent stressful events for the animals, which have a direct effect on reducing appetite and feed intake. Appetite is regulated by various factors as shrimp has a highly developed and sensitive sensory system, and appetite messages are known to reduce stress messages. In this context, modulating shrimp appetite can have a positive effect on stress reduction. A trial was conducted to evaluate the effect of a neurosensorial feed additive (NFA) on shrimp stress reduction through feed intake.

Material & Methods

During 8 weeks, 900 Pacific white shrimp (*Litopeneaus vannamei*) with an average initial weight of 0.5g were divided into 18 ponds of 220L in brackish water with 15 ppt salinity. Faecal matter was siphoned daily, and 60% of the water was changed every third day. The balanced feed used had a protein content of 35% and 7% lipids. The feed was distributed to the shrimp 4 times/day. The NFA was applicated at a 3kg/T rate. Each treatment was replicated 4 times. Feed intake rate, live weight, survival rate, and total biomass were measured at the end of the experiment (week 8). To measure stress reduction, an acute stress induction protocol was started at week 4.

Results

The use of the NFA led to a global improvement of survival rate, from 44 to 62% (Fig.1). After the stress induction protocol, it was observed that immobility time was reduced from 25 to 5 seconds with the NFA. These results suggest that a neurosensorial product have a beneficial effect on stress and mortality reduction in shrimp

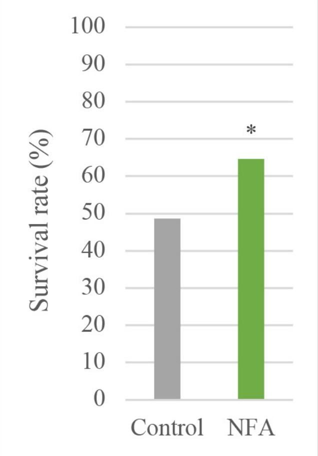

Fig.1 Final survival rate at week 8 *p<0,05

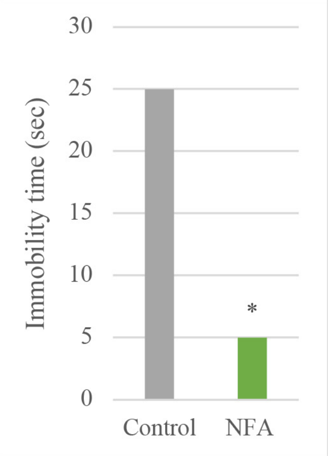

Fig.2 Average immobility time after acute stress in week 4 *p <0,05

EVALUATION OF A NEUROSENSORIAL FEED ADDITIVE ON Litopeneaus vannamei SHRIMP FEED INTAKE WITH LOW-FISHMEAL FEEDS

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Introduction

One of the biggest challenges of shrimp aquaculture industry is the reduction of the use of fishmeal and fish oils, with balanced feed called "low-fishmeal (LFM)" feeds. These alternatives often fit well to animals from a nutritional point of view, but tend to under look the taste and attractivity aspect, in which fishmeal plays a huge role. The present study aimed to evaluate the effect of a neurosensorial feed additive (NFA) on feed consumption of shrimp fed with HFM (High-fishmeal) and LFM feeds.

Material & Methods

During 8 weeks, 900 Pacific white shrimp (Litopeneaus vannamei) with an average initial weight of 0.5g were divided into 18 ponds of 220L each in brackish water with 15 ppt salinity. Faecal matter was siphoned daily, and 60% of the water was changed every third day. The balanced feed used had a protein content of 35% and 7% lipids. The feed was distributed to the shrimp 4 times/day. Diet HFM contained 20% of fishmeal whereas diet LFM contained 10% of fishmeal and 10% of soy protein. Each diet was tested with the inclusion of the NFA (1,5 kg/T) and a control, and each treatment was replicated 4 times. Feed intake rate, live weight, survival rate, and total biomass were measured at the end of the experiment (week 8).

Results

Results suggest that the NFA improves low-fishmeal feeds consumption, with similar levels to those of high-fishmeal feeds.

The NFA could be a good solution to implement an efficient strategy to replace fishmeal in shrimp feeds.

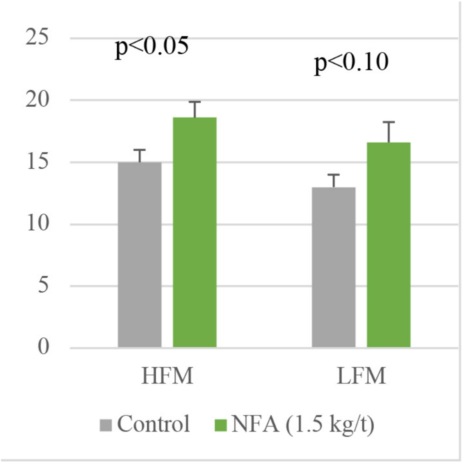

Fig 1. Total feed intake after 8 weeks

EFFECT OF A PROPRIETARY BLEND OF ESSENTIAL OIL BIOACTIVES ON STRESS RESPONSES OF *Salmo salar*

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Introduction

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 an antistress functional feed additive (AFFA) mainly based on proprietary blend of essential oil bioactives, on the stress induced by handling and crowding subsequent stages.

Material & Methods

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 AFFA; 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 AFFA and crowded to a density of 85Kg/m^3 to reproduce typical conditions of salmon transportation. These containers contained the same concentration of AFFA as the original tanks. Fish stress responses were measured through: behavioral observations, cortisol levels at 5 different times following the AFFA 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.

Results

Regardless of the essential oil bioactives 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 an AFFA for fish sedation at 20-ppm concentration repeated every 2 hours.

THE IN-POND RACEWAY SYSTEM (IPRS) TECHNOLOGY: PROMOTING THE USE OF HIGH VALUE FEEDS AND INGREDIENTS FOR OPTIMZING PRODUCTION AND PROFIT

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The United States Soybean Export Council (USSEC) has worked for over 35 years with the aquaculture industry worldwide to promote a profitable, responsible, feed-based aquaculture industry while specifically promoting the use of United States soy products in aquaculture feeds. Though the primary target of the program is to work with feeds, an important aspect of the program is to help farmers with improving their aquaculture production systems, including introduction of better technology approaches.

Use of diets manufactured with high quality ingredients (macro and micro) is fundamental to success and profitability in modern aquaculture. However, nutritional strength and high levels of digestibility by themselves are not enough to assure sustainability or profitability. Animal health and high survivorship to market target weight is the most critical element to any successful aquaculture business.

USSEC has supported the development and adoption of the In-pond Raceway System (IPRS) technology, an alternative to traditional pond culture, which allows more efficient and productive use of water volume. IPRS operational principles combine to allow managers to increase annual yields 200-300% with no water exchange. IPRS provides the system manager with the necessary tools and fish culture environment to achieve high levels of survivorship, nutrient digestibility and retention leading to profitable and therefore sustainable operations. After many years in development and commercial application, IPRS has been increasingly adopted globally as a highly successful, predictable, and sustainable strategy for pond production.

A core of this technology's success is the use of high-quality feeds and good feeding approaches that balance a low feed conversion and fast growth rate to optimize the use of the production unit, which helps to promote a lower cost per unit production. This presentation will discuss the linkage of feed and ingredient quality and how it helps to optimize the use of the IPRS technology.

TRANSPOSING CIRCULAR ECONOMY PRINCIPLES TO AQUACULTURE

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Moving from a linear to a circular economy (CE) is perceived a way to reduce the environmental impacts of human activities, including those of (blue)food productions, by closing materials and energy loops, and by implementing transformational changes in production and consumption. CE and its principles are still underexplored in aquaculture, although its fundamentals (i.e., reduce, reuse, recycle, recover) have already been largely applied. A recent study presented a set of 5 principles applicable to food and non-food systems to guide biomass use towards a circular bioeconomy (Muscat *et al.*, 2021): safeguarding and regenerating the health of our agroecosystems; avoiding non-essential products and the waste of essential ones; prioritizing biomass streams for basic human needs; utilizing and recycling by-products of agroecosystems; and using renewable energy while minimizing overall energy use. Most of the examples provided by the authors were based on terrestrial food production systems (i.e., agriculture and livestock), questioning therefore their applicability to the aquaculture sector and their novelty in comparison to well-known sustainability schemes.

The main objective of this study is to transpose the 5 principles developed by Muscat et al. (2021) to the field of aquaculture, identifying the implications of a transition towards more circularity in aquaculture and highlighting current knowledge gaps to initiate this transition. We performed a literature review to address several questions emerging from the CE paradigm including: Which aquaculture forms and what farming practices can safeguard and regenerate the health of our aquaecosystems? What makes aquatic products essential, and where and for whom are they essential? Are all aquaculture products equally essential? Given the use of human-edible ingredients in aquafeed, what is the net contribution of aquaculture products to human food provision? How can the aquaculture sector be used as a sink to recycle by-products from the food system and close nutrient loops at farm, or higher levels (e.g., territory)? What are the main levers to reduce the energy footprint of aquaculture products?

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THE USE OF RAPID MOLECULAR TECHNIQUES FOR THE IDENTIFICATION OF *E. aeneus* AND *E. marginatus* FROM GREEK CATCHES

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Introduction

Fish play an important role in the Mediterranean diet, providing high-quality protein content, vitamins, minerals, and large amounts of ω-3 polyunsaturated fatty acids. Although, aquaculture products cover a large percentage of fish production, consumers still have a preference to wild catches. *Epinephelus* is a genus of predatory fish, including 89 recognized species, found in seas throughout the world. They are among the highest priced species in fish markets, and only a few species are used in aquaculture (Heemstra and Randall, 1993). Two of the most common representatives in Greece are the dusky grouper *E. marginatus,* a reef-associated species distributed in the Mediterranean Sea, Atlantic and Indian Ocean and the white grouper *E.aeneus,* a demersal species distributed in the Mediterranean Sea and the Eastern Atlantic Ocean (Fishbase). According to the IUCN Red List of Threatened Species, *E. marginatus* has been classified as "Vulnerable" and *E. aeneus* as "Near threatened" species. Both species are distributed in Greek markets and restaurants, with high demand especially during the summer period. The fact that Greek Small-Scale Fisheries (SSF) often work seasonally, fish stocks decline and imported representatives of *E. marginatus* and *E. aeneus* are also traded in the Greek market, raises the question of authenticity, traceability and proper labeling of *Epinephelus* fish from Greek catches. In addition, incidents of Grouper fillets substitution by cheaper fish species, such as the Nile perch (*Lates niloticus*) or the wreck fish (*Polyprion americanus*) have been reported in Spain (Asensio et al., 2008). Molecular techniques based on DNA, e.g., COI barcoding, are currently being used for accurate and robust species identification, by using samples of any form: raw, cooked, processed, frozen. However, sequencing-based methodologies can be time consuming and not applicable for rapid food controls in the market. For the discrimination between closely related species or populations within species, data could be drawn from mtDNA regions. In this study, regions of mtDNA from *E. aeneus and E. marginatus*, captured in the Greek seas, as well as their imported counterparts sold in the Greek markets, were sequenced. Barcodes were used to identify the species and data inferred from other gene sequences were tested by non-sequencing based techniques such as PCR-RFLPs, multiplex PCR, HRM analysis, in order to establish a fast and reliable method for the identification of *E. aeneus and E. marginatus* from Greek catches.

Materials and Methods

The specimens examined were: (a) wild *E. aeneus* (40 samples) and *E. marginatus* (35 samples) caught in the Greek seas, FAO subareas 37.2 (Ionian Sea division 37.2.2) and 37.3 (Aegean and Cretan Seas), (b) imported samples labelled as *E. aeneus* (10 samples) and *E. marginatus* (11 samples), purchased from fish markets and supermarkets, (c) cooked samples collected from restaurants (3 samples of *E. aeneus*) and (d) *L. niloticus* and *P. americanus*. When available, fish were identified according to their morphological characteristics and categorized in groups by their origin. Total DNA was extracted from different tissues (muscle, liver, fins) using either NucleoSpin® Tissue and/or NucleoSpin® Food Macherey-Nagel, according to the manufacturer's instructions. For barcoding, a COI fragment of 648 bp was amplified using universal primers (Ward et al., 2005). For the amplification of mtDNA regions new primers were designed with Geneious Prime® 2022.2.1 Biomatters. Sequences were identified with Blast l, compared and aligned with sequences from GENBANK and BOLD databases and aligned with Clustal Omega, Molecular Systems Biology. For both species (a) PCR-RFLP analysis was performed (*E. aeneus*, 3 fragments and *E. marginatus* 1 fragment), (b) Multiplex PCR, (4 fragments were amplified for *E. aeneus* and 6 for *E. marginatus)* and (c) High-Resolution Melting (HRM) analysis (fragments of 80-120 nt for COI, 16sRNA *cytb* and *D-loop*).

Results

By barcoding, all samples of fish from Greek catches and 8 samples of imported fish from Senegal, Tunisia and Spain were identified as *E. aeneus*. Two imported samples from India were identified as *E. undulosus* and *E. poecilonotus*. Respectively all fish morphologically classified as *E. marginatus* were confirmed by barcoding. The same samples were analysed by PCR-RFLP but only an 875bp fragment of ND1-ND2 region digested with SacI could distinguish between *E. aeneus* compared to *E. undulosus* and *E. poecilonotus*. Multiplex PCR analysis of 4 fragment for *E. aeneus* showed a pattern that could distinguish between *E. aeneus* compared to *E. undulosus* and *E. poecilonotus* but not between domestic and imported specimens. Respectively the analysis of 6 fragments for *E. marginatus* showed no distinction between Greek and imported specimens. HRM analysis using different mtDNA regions showed that: (a) both COI and *cytb* fragment were species indicative, but not applicable for the discrimination between Greek and imported specimens, (b) 16s and CR regions were indicative for both fish species and population identification.

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Conclusions

Fishing plays a crucial role in employment and economic activity (trading, catering, tourism) for Greek coastal communities (FAO 2020). As many Mediterranean fish populations *E. aeneus* but mainly *E. marginatus* populations are in deep crisis, therefore sustainable fishing and conservation of the stocks are of high importance. By the assessment of authenticity and traceability of fish products not only consumers are protected, but also fish stocks are surveyed. In this study, the molecular identity of each species has been determined, even if the fish have been processed (exfoliated, fillet or cooked) thus ensuring, originality and authenticity of Greek fishery products. Our data show that HRM is a promising methodology that can be used as a rapid tool against fish mislabeling and phenomena of fish fraud.

Acknowledgements

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INDIVIDUAL TELEMETRY OF EUROPEAN SEABASS IN SEACAGES REVEALS BEHAVIOURAL AND POSITIONAL PATTERN CHANGES INDICATING FOOD ANTICIPATORY ACTIVITY

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Introduction

Feeding behavior can be described through a variety of factors, involving anticipation, stress, health, and activity. Measuring each one can be hard, because they are interdependent or underly circadian clocks.

Accurately describing the behavior preceding feeding is difficult but can lead to improvements on feeding schedules to optimize nutrition usage. We therefore investigate the nature of Food Anticipatory Activity (FAA) displayed in sea caged European seabass, especially with respect to diving behavior and acceleration activity. Our work expands the aquatic knowledge in FAA and is a step towards precision aquaculture with accurate feeding schedules independent from internal rhythm patterns.

Materials and methods

Around 10 thousand individuals of European seabass (*Dicentrarchus labrax*) with an estimated weight of 450 grams each were held in a sea cage of 40m diameter and 9m cage depth at an aquaculture facility off the island Crete, Greece. The feeding schedule consisted of once a day manually feeding between 8am and 10am local time. 24 fish were prepped with a tag with two transmitters, which sent depth and GPS position every 5 minutes, and alternately temperature and activity using a 3-axis accelerometer. Three receivers positioned around the cage collected positional, temperature and activity data from the transmitters between 28.05.2021-06.06.2021 to analyze positional and behavioral changes in the feeding process. We split the entire water column of the sea cage into three segments: The upper water column ranged from 0-3m depth, the middle water column ranged from 3-6m depth, and the lower water column ranged from 6-9m depth.

Results

Figure 1 shows an almost identical relative distribution of recorded fish positions between the three water columns. The day-night distribution was around 75% to 25% across the water columns, if the different twilights (official, nautical astronomical) are counted in as day. The difference in time spent in each water column during and before the feeding window (Fig. 2, red and green chunk in the first vertical bar) hinted at: 50% of the time where the seabasses were in the upper water column (0-3m) was before and during feeding, whereas for the other two water columns, less time was spent there before and during feeding. When residing in the middle water column, around half of the fish position pings were recorded after 2pm. Figure 3 illustrates 24-hour periods where activity was higher during day and fell lower during night with the twilights as transitional periods. On a weekly scale, we noted that activity followed the temperature curve, and slightly increased towards the end of the experiment (see Fig. 3).

Discussion and conclusion

The day-night distribution of 75%-25% described in Figure 1 was not surprising, since the tags transmitted in an even interval combined with long days in Crete. For example, the night stretching from the astronomical sunset on the 26.05.21 to the astronomical sunrise on the 27.05.21 is 6 hours 11 minutes long, which is approximately 25% of a 24-hour day, verifying our data quality. We were not able to detect a significant positional change throughout the day in Fig. 1, neither water column-wise nor timewise, suggesting that the fish were using the entire water column.

Due to the discrepancy between the different water columns just around and before the feeding time, we argue that the E. seabass was anticipating food and swimming up to the upper water column to prepare for food intake, which falls under FAA. We conclude that the cyclic regular feeding method created a habit in the fish to await food and therefore to swim up and be “prepared”. It appears therefore as the FAA was also at least correlated with the time of day, since we could not observe any FAA during nighttime.

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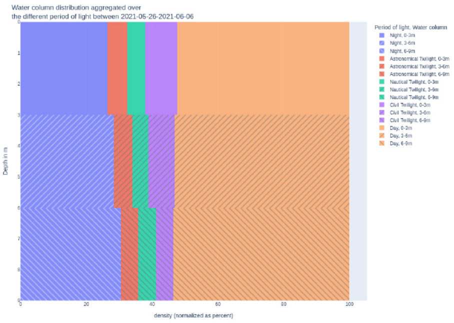

Figure 1: The aggregated density of the E. seabass throughout the water columns colored by the light period.

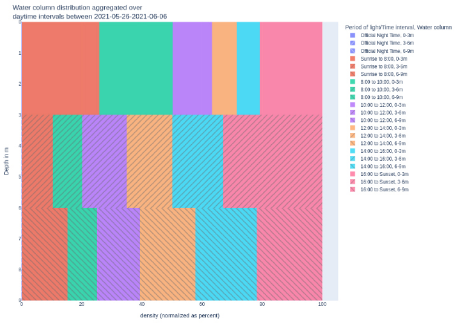

Figure 2: The aggregated density of the E. seabass throughout the water columns colored by different time intervals.

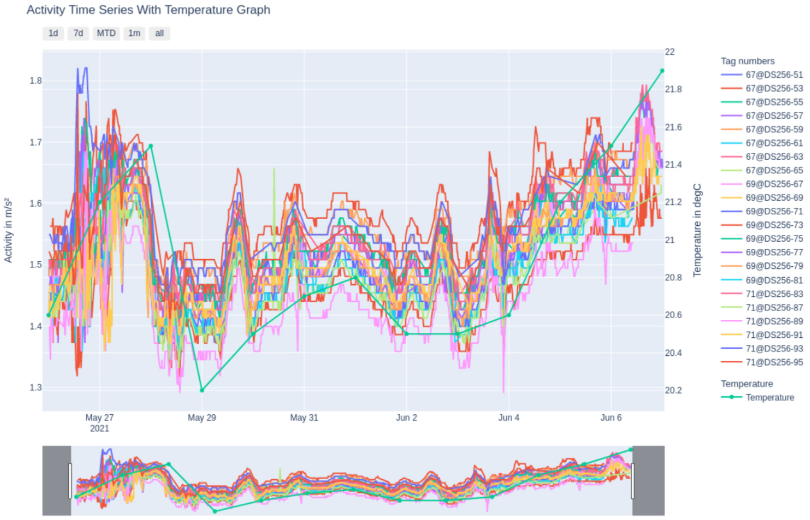

Figure 3: Activity of the tagged fishes during the experiment with a temperature line as overlay (green).

Higher activity throughout the first week of June was expected, since we know that temperature correlates with the activity of fish, as Figure 3 illustrates. It is remarkable that individual fish tend to stay in their hierarchy when sorted after “most active” to “least active”, which we argue is due to the different physiological traits the individuals have. The spike in the beginning of the experiment with the high complex interweaving of the lines may be due to the initial habituation period where the fish had not established activity profiles yet. It remains to be discussed more thoroughly though.

Acknowledgments

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AQUACULTURE OPPORTUNITY AREAS: A NEW APPROACH TO U.S. AQUACULTURE DEVELOPMENT

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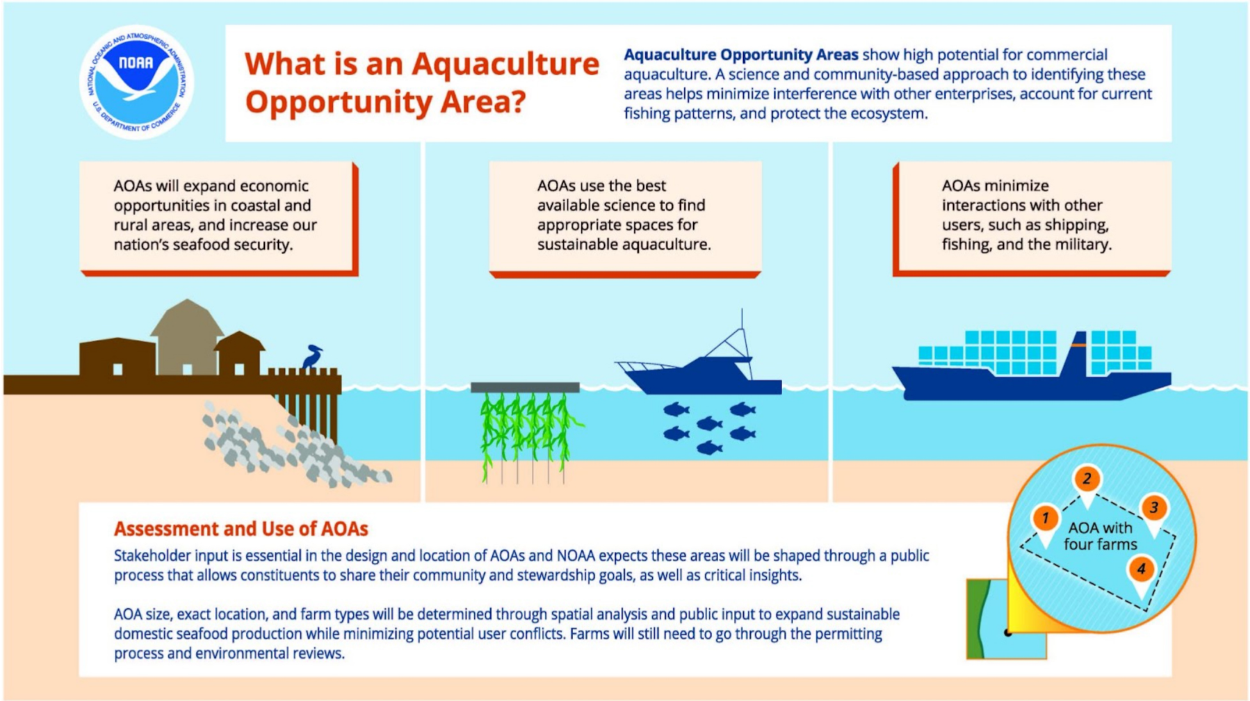
With a new vision and strategic plan to support aquaculture development, NOAA is leading a shift-change in U.S. aquaculture development through the identification of Aquaculture Opportunity Areas (AOAs). The search for AOAs in U.S. waters is a planning process, not a regulatory process, to identify defined 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.

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. AOAs are intended to minimize interactions of aquaculture development with other ocean uses and important environmental resources, maintaining NOAA's commitment to ocean stewardship. NOAA will provide information on our progress towards identifying the first two geographic areas containing locations suitable for commercial aquaculture, drafting the PEIS for each of those two areas, and the opportunities for stakeholder input into the process.



What is an Aquaculture Opportunity Area?

Aquaculture Opportunity Areas show high potential for commercial aquaculture. A science and community-based approach to identifying these areas helps minimize interference with other enterprises, account for current fishing patterns, and protect the ecosystem.

AOAs will expand economic opportunities in coastal and rural areas, and increase our nation's seafood security.

AOAs use the best available science to find appropriate spaces for sustainable aquaculture.

AOAs minimize interactions with other users, such as shipping, fishing, and the military.

Assessment and Use of AOAs

Stakeholder input is essential in the design and location of AOAs and NOAA expects these areas will be shaped through a public process that allows constituents to share their community and stewardship goals, as well as critical insights.

AOA size, exact location, and farm types will be determined through spatial analysis and public input to expand sustainable domestic seafood production while minimizing potential user conflicts. Farms will still need to go through the permitting process and environmental reviews.

Figure 1. What is an Aquaculture Opportunity Area?

ANTIVIRAL FUNCTION OF NKEF AGAINST VHSV IN RAINBOW TROUT

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Introduction

Natural killer enhancing factor (NKEF) belongs to the peroxiredoxin family of proteins, a group of antioxidants that has been extensively studied in mammals. Recently, we identified NKEF in the immunoprecipitated proteome of rainbow trout red blood cells (RBCs) exposed to viral hemorrhagic septicemia virus (VHSV). In the present study, we evaluated the role of NKEF in the antiviral response of rainbow trout against VHSV by examining the expression profile of NKEF in VHSV-exposed RBCs and rainbow trout gonad-2 (RTG-2) cell line.

Material and methods

Through silencing and overexpressing NKEF, using siRNA or a plasmid vector, respectively, we determined the effect of NKEF in the infectivity of VHSV, in rainbow trout RBCs *ex vivo* culture, RTG-2 and EPC cell lines. We also evaluated, by means of qPCR, the implication of NKEF in the modulation of the antiviral immune response.

Results

We found a correlation between decreased VHSV replication and increased NKEF expression in RBCs exposed to VHSV, however, this was not found in RTG-2 cells where *nkef* transcripts remained almost unchanged while the infection increased with the time. In addition, siRNA silencing of the *nkef* gene in rainbow trout RBCs and RTG-2 cells resulted in increased VHSV replication. We also found a correlation between *nkef* gene silencing and a decrease in the expression of genes related to type 1 interferon (IFN1) pathway. These findings indicated that NKEF is involved in the antiviral mechanisms of rainbow trout RBCs against VHSV and thus support its antiviral role and implication in the modulation of the immune response. Finally, overexpression of NKEF in an EPC cell line significantly reduced VHSV infectivity and was coupled to an increment in IFN1-related genes. Therefore, we could conclude that NKEF may be a potential target for new therapeutic strategies against viral infections.

ALIEN SPECIES ASSOCIATED TO COMMERCIAL BIVALVE TRANSLOCATIONS

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Introduction

Council Regulation (EC) N. 708/2007 concerning use of alien and locally absent species in aquaculture, aims to assess and minimize the possible impact of these species and associated non-target ones on aquatic habitats. It provides indication to Member States to develop procedures for risks analysis and contingency plans for mitigation. However, the traceability data for bivalve introductions and translocations are currently lacking and must be improved not only in Italy, as well as in whole Europe.

Aquaculture practices include importation of seeds and live animals from different countries and their translocations within European marine waters; these practices represent one of the main vectors of the biological invasions from alien species, with increasing risks for farming activities, food and consumers' safety, as for environmental and biodiversity.

In Italian aquaculture, shellfish cultivation plays a relevant role, and it is mainly based on 4 species: the native Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819), the alien Manila clam *Ruditapes philippinarum* (Adams and Reeve, 1850), the native grooved carpet shell *Ruditapes decussatus* (Linnaeus, 1758) and the alien Pacific oyster *Magallana gigas* (Thunberg, 1793) (see Salvi & Mariottini, 2016), previously known as *Crassostrea gigas* (Thunberg, 1793). Native flat oyster *Ostrea edulis* (Linnaeus 1758) production declined in all Europe and in Italy too; alien blue mussel *Mytilus edulis* (Linnaeus, 1758) is also locally farmed with small productions in Sardinia Region.

Considering the current scenario, the aim of the present review was to draft an updated synthesis of literature data on alien species associated to translocations of commercial bivalves. Collected data will be useful to improve the traceability system for farmed bivalves in Italian marine waters, with particular reference to non-target species spread and diffusion.

Materials and method

Google Scholar, Scopus and WoS (Web of Science) were used for this bibliographic research with the following parameters: years from 2000 to May, 2022; selected topics and/or keywords "shellfish introduction", "shellfish translocation", "shellfish aquaculture", "oyster", "mussel", "clam", "Pacific oyster", "*Magallana gigas*", "*Crassostrea gigas*", "Manila clam", "*Ruditapes philippinarum*", "blue mussel", "*Mytilus edulis*", combined with "alien species", "hitchhikers", "associated NIS", "pathogens" and "virus". *C. gigas* was also used for bibliographic research since it was the valid species name until 2016.

For the present review, only papers concerning shellfish species cultivated in Europe were considered; the bibliography of collected papers was also used to integrate our data.

All the species names were carefully checked, confirmed, and updated on WoRMS - World Register of Marine Species (https://www.marinespecies.org/), as to describe the original range of species distribution.

Results and discussion

After removal of duplicates, 48 scientific papers were collected and analyzed. From literature synthesis, overall, 147 taxa (genus or species) have been identified for Bacteria, Chromista, Plantae and Animalia, plus three viruses or variants, already introduced though shellfish farming, or potentially suitable for introduction or translocation by commercial species of oysters, clams and mussels.

These alien species may affect (i) directly the cultivated bivalves (pathogens and parasites), (ii) the environmental quality (potentially toxic phytoplankton) and/or (ii) other marine species, with impacts on biodiversity.

Literature data herein collected also showed that many alien species introductions may have been facilitated by oyster, clam and mussel farming activities, although their further diffusion have then improved by other vectors.

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Among the cultivated bivalve species herein considered, oysters have been identified as the major vector of introduction for many alien species, with particular reference to Pacific oyster *M. gigas*. Despite its faming is now limited to the 1% of the Italian production, it has been growing since 2018 (FAO, 2022), representing a promising sector of national shellfish aquaculture (Marino et al., 2020). Therefore, it should be carefully monitored and managed to avoid the risks of new introductions and spread of associated alien species.

From this literature collection clearly emerges the necessity to improve the traceability system in bivalve aquaculture, especially for shellfish translocations: main efforts should be made at national and European level on the definition of biosecurity plans for non-target species, such as those already applied in Ireland, Australia, New Zealand.

Improving the knowledge on non-target species associated to shellfish aquaculture is therefore necessary to further implement the Council Regulation (EC) N. 708/2007, but also to support the monitoring activities referred to Descriptor 2 of the Marine Strategy Framework Directive.

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"SOY PRODUCTS AS SUSTAINABLE INGREDIENT IN AQUA DIETS; WITH SPECIAL REFERENCE TO U.S. SOY"

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From a global perspective, sustainability is a topic of much importance. The UN Sustainable Development Goals (SDGs) were set by the 193 member states of the United Nations in 2015 with a timeline to achieve the goals by 2030. Sustainability in the aquaculture value chain begins with the raw materials used in the feed. Raw materials represent the cornerstone for fish health and growth, and therefore ultimately for the fish farming success.

Historically, the base raw materials for aquaculture feeds have been fishmeal and fish oil, which is sourced from small forage fish, such as anchovies, sardines and capelin. Every year about 19 million tons of wild fish – some 20% of the total quantity caught around the world – are rendered in to fish meal and fish oil. Increasing use of forage fish is unsustainable and, because an additional 37.4 million tons of aquafeeds will be required by 2025, alternative protein sources are needed (Hua K et al. ,2019).

The total production of aquafeeds for all aquaculture species is projected to increase by 75% from 49.7 million tons in 2015 to 87.1 million tons in 2025 (Hua K et al., 2019). Analysts predict that the increased demand of aquaculture for fish meal and fish oil will outstrip the supply of small forage fish, by 2037.

Consistent with available evidences from a range of research trials, plant proteins can replace fish meal either in part or completely when certain dietary recommended conditions are respected. This is relatively well accepted for omnivorous species but less so for carnivorous fish despite solid research results for specific species (Kaushik et al., 2004, Dani, 2018; Kotzamanis et al., 2020, Zapata, 2021). As soy ingredients become an increasingly significant ingredient, it is only natural to focus on sustainability and reducing environmental impact. Soy crops are often connected to environmental issues such as deforestation and other unwanted Land Use Change (LUC) issues. Retailers, NGO's and consumers in Europe are increasingly concerned that this conversion leads to CO_2 emissions, land degradation and biodiversity loss.

The LCA (Life Cycle Assessment) method was used to evaluate the environmental impact of a soybeans throughout their entire life cycle. The method assesses every stage of the production, processing and use of soy, from raw material, packaging and transport to retail, consumption and waste-processing. This work was carried out by Blonk consultancy (The Netherlands) for USSEC.

Results showed that the carbon foot print (in kg CO_2-eq/kg product) - without taking the estimated LUC (Land Use Change) into consideration - averaged 0.37, 0.34 and 0.46 kg CO_2-eq/kg soybeans for the USA, Argentina and Brazil respectively. For all three countries cultivation accounted for more than 50 % of the CO_2-eq. production but there were major differences among the three countries in the effect of transport and processing with Brazil having a much larger impact of transport. Including LUC in the calculations resulted in pronounced differences in carbon foot prints with averages of 0.39, 5.56 and 5.75 kg CO_2-eq/kg soybeans for the USA, Argentina and Brazil, respectively. This clearly emphasized the importance of the cultivation methods and the differences among the three major regions in managing natural resources in the production and marketing of soybeans. The U.S. system being characterized by a rotational production in which land conservation plays a major role, and where the use of cropland has decreased by an average of more than 600,000 ha/year over the past 35 years. Over the same period, in the USA, forest land has increased by 2.1 million ha while this has decreased in South American countries.

These data support clearly the potential of soy protein as a sustainable alternative to other protein sources used in aqua production. Replacement of classical protein sources such as fish meal or animal proteins, but also a wide range of plant protein, can be accomplished through the use of responsible and sustainable soy protein. Of course, this requires the establishment of official organization supervising and certifying the sustainability of soy production. Such mechanisms are in place for US soy production, but need to be developed for soybeans of other origin.

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PRODUCTION OF FUNCTIONAL AND AROMA COMPOUNDS FROM FISH WASTE AND BY-PRODUCTS USING SELECTED MICROORGANISMS

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Introduction

The fish processing industry has experienced significant growth over the last decades due to a rapid increase in urbanization, industrialization and consumer request. This process has contributed to generate more and more waste and by-products that are ususually discarded or minimally recovered. In order to promote a circular economy, these sidestreams like fish heads, skin, bones, spines and viscera, are still a source of low value compounds that can be turned into added value compounds through biotechnological process. Therefore, the selection of microroganisms capable of growing and fermenting these substrates is of croucial importance (Marti-Quijal et al., 2020; Ideia et al, 2020).Microbial fermentation or release of enzymes, such as proteases and lipases, can generate functional peptides, lipids, flavouring compounds, and chitosan. The aim of this study, performed in the framework of the European project "NewTechAqua", was to select and exploit safe microroganisms capable of fermenting fish waste and by-products and producing added value molecules or enzymes.

Materials and Methods

Different strains of yeast (*Yarrowia lipolytica*, *Debaryomyces hansenii*) and bacteria (*Lactiplantibacillus plantarum, Lacticaseibacillus paracasei, Bacillus subtilis, Pseudomonas* spp.) were screened for their enzymatic activities (proteolysis and lipolysis) using skim milk, gelatin plate, and spirit blue agar, respectively (Abbas et al., 2020; Cotârleț, Stănciuc & Bahrim, 2020; González et al., 2020). Selected microorganisms were then incubated for 72h in a solution containing 10 g of waste (fish bones, shrimp shells, viscera and blood) and 10 ml of glucose 30%. After fermentation, the supernatants containing protein hydrolysates were collected by centrifugation (4000 rmp for 10 min), quantified using Bradford and OPA assaies, and assessed for their antioxidant (DPPH, ABTS, TBARS), anti-hypertensive (anti-ACE assay) activities, and the ability of inhibiting pathogenic bacteria (*Escherichia. coli* EC15992, *E.coli* 555, *Salmonella* SE155, *Listeria monocytogenes* SCOTT A and *L. monocytogenes* 56LY) (Hong et al., 2014; Gottardi et al., 2014). Moreover, the production of flavouring compounds was evaluated by SPME/GC-MS technique. Protease production was evaluated according to Si et al., (2018).

Results

Among the microorganisms tested *in vitro*, all the strains of *Y. lipolytica* showed good proteolytic and lipolytic activities, followed by *Bacillus* spp. strains. The most promising strains were then incubated in a solution containing fish waste and by-products. *Y. lipolytica* generetaed more functional peptides. In fact, fresh hydrolysate obtained from *Y. lipolytica* 2 and 4 were those with the highest peptide content and the strongest radical scavenging activity (up to 86 % for DPPH, and 1.6 mg/ml equivalent to Trolox for ABTS). Moreover, the same samples hinhibited the growth of some strains of *E. coli*, *Listeria monocytogenes* and *Salmonella* spp.. Growth of *Y. lipolytica* also generated several volatile molecules, especially aldheydes, that could be exploited as food aroma. Fermentation obtained with Lactic acid bacteria and bacilli produced proteases that could be extracted and applied in the food industry.

Conclusion

This work has shown the relevance of fermentation as a useful tool to valorize fish waste and by-products, reducing their environmental impact and giving them an added economic value.

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EFFECT OF THE PROBIOTIC *Lactococcus lactis* ON THE MICROBIAL COMPOSITION IN THE WATER AND THE GUT OF FRESHWATER PRAWN (*Macrobrachium rosenbergii*) CULTIVATE IN BIOFLOC

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Introduction

The importance to study the intestinal microbiota of the prawns contributes to the knowledge of the resident bacteria with probiotic capacities. In recent years, the search for new sustainable aquaculture technologies has allowed the development of biofloc culture systems, in which microbial oxide reduction processes are carried out through the addition of a carbon source (Ferreira et al., 2015). Therefore, during the present study, the diversity and abundance of the microbial community in the culture water and the gut of the prawns cultured in biofloc and with the addition of the probiotic *Lactococcus lactis* were evaluated.

Materials and methods

For the study, 240 post-larvae of prawns were placed and distributed in 12 containers of 250 L capacity (Emerenciano et al., 2012). During 127 days of culture, an experiment was carried out with four treatments with three replicates (Emerenciano et al., 2012). Treatments: T1 consisted of prawns cultured in biofloc; T2 prawns cultured with biofloc and the addition of two doses of *L. lactis* at a concentration of 1×10^7 CFU ml^{-1} incorporated through *Artemia*; T3 corresponded to the addition of two doses of the probiotic at the same concentration; T4 cultured in clear water. Survival and weight were measured to determine individual growth. To determine the bacterial diversity in water and gut samples were taken and performed using MOTHUR (Schloss et al., 2009) to calculate five metrics to assess bacterial communities, including the number of observed OTU's, Shannon diversity index, and Inv-S estimators.

Results

The survival and the final weight of the prawns in the treatments BFT (100 ± 0), BFTPROB (100 ± 0) and PROB (93 ± 1.3) was significantly higher than that observed in the prawns in the CONT (80 ± 4.1) treatment. The highest weight gained (g) and SGR were obtained in the prawns subjected to the BFTPROB (3.3 ± 0.7) treatment, which was significantly higher than that observed in BFT (3.1 ± 0.8) and PROB (3.2 ± 0.5), while the lowest results were obtained with the CONT (2.8 ± 0.8) treatment, indicating a higher efficiency when incorporating biofloc and probiotics in the culture systems. Water quality parameters during the study were generally within tolerance ranges to maintain efficient growth rates in the prawns (Avnimelech, 2012). Respect the metagenomic analysis, the lowest number of OTU's and richness index (Chao) was PROB in the intermediate phase of the water samples (240 and 280.94 respectively) and the treatments with the highest number of OTU's and richness were BFT in the intestine (300 and 357.36 respectively) and PROB in water in the final phase (300 and 358.89 respectively). The diversity of the bacterial communities' indices per experimental stages were similar in values, except for the CONT 17.70 (17.86– 17.53), it was the lowest. The results of the metagenomic analysis indicate that the microbial community of the water in the biofloc/probiotic treatments samples were represented by 17 populations of taxonomic classes (Figure 1 and 2).

Discussion

According to the results, the survival and growth of the prawns of the BFTPROB treatment were significantly higher compared to the CONT prawns; this can be attributed to the action of the microorganisms (heterotrophic bacteria, phytoplankton, zooplankton, ciliates, rotifers, nematodes, and copepods) present in the biofloc as a source of natural protein in situ and available 24 h a day (Emerenciano et al., 2013). It is possible to consider that an ecologically efficient way to maintain water quality in general and, particularly, the concentration of nitrogenous metabolites during a commercial culture is the use of bacterial bioflocs, which contribute to maintain the concentration at values lower than those toxic to the prawns. The results about the 17 classes indicate that there is a direct relationship between the bacteria present in the water and those found in the gut samples, it is known that Proteobacteria are a microbial group that is responsible for the nutrient recycling process and mineralization of organic components in aquatic systems (Cardona et al. 2016). On the other hand, the absence of the probiotic *L. lactis* as part of the dominant microbiota in the PROB and BFTPROB treatments is probably due to it was displaced by the biofloc bacterial community (Cardona et al., 2016; Cienfuegos et al., 2018; Deng et al., 2019).

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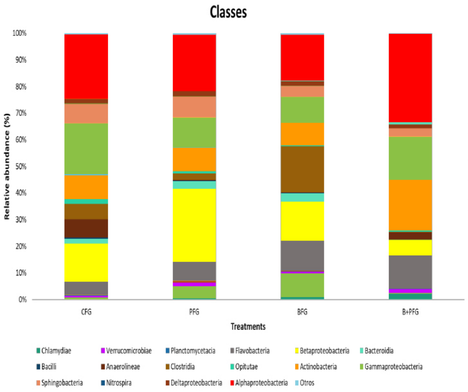

Fig 1. Average relative abundance (%) of the classes of bacteria in the gut samples of the experimental prawns.

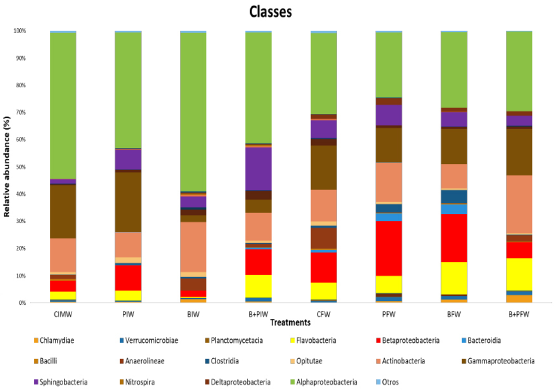

Fig 2. Average relative abundance (%) of the classes of bacteria present in the water samples from the experimental treatments.

Conclusions

This study expanded the knowledge about the diverse bacteria that develop in the biofloc and when probiotics are added, because in most of the investigations only phyla Firmicutes, Proteobacteria and Actinobacteria have been reported as dominant; with the results of this research 17 classes were identified within which Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria, Flavobacteria, and Clostridia were considered the most abundant. The probiotic *L. lactis* was not observed as part of the dominant microbiota in the treatments where it was added, this is probably because it was displaced by the microbial community of the biofloc. Despite this, significant differences were observed in the growth and weight of the prawns; survival was higher in the treatments with respect to the control.

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EVALUATION OF THE EFFECT OF LETTUCE-BASED DIETS ON *Paracentrotus lividus* LARVAL DEVELOPMENT

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Introduction

Depletion of natural stock of edible sea urchins turns them into target species for aquaculture practices that could secure high-quality seeds, juveniles and adults 1. Positive results were obtained using vegetable discards as diet for the gonad enhancement of Paracentrotus lividus (Vizzini et al., 2019), the most exploited Mediterranean sea urchin. Here, we analysed the embryo-larval development in offspring from adults reared under five diets (four based on Lactuca sativa discards and one on macroalgae), while the Integrated Toxicity Index (ITI, Morroni et al., 2016) was applied to assess diet quality in terms of normoformed/abnormal larvae. Finally, Stable isotope analysis were conducted to evaluate each diet assimilation.

Materials and Methods

Four diets were made with *L. sativa* discards of (86%) and to lesser extent (10%) of one of four seafood meals (fish, Diet-F; krill, Diet-K; mussels, Diet-M; and fish discards, Diet-D). A fifth diet was composed of 48% of both *Ulva* sp. and *Laminaria* sp. (Diet-A). 110 adults of *P. lividus* were collected at Santa Marinella (Italy) and transported to the LESA Lab. of University of Rome "Tor Vergata". 75 females were allocated into 15 tanks (3 for each diet) of a recirculating aquaculture system and fed *ad libitum* for five months. Then, spawning was inducted and the eggs collected and fertilized. After 48h in a thermostatic chamber, larvae were fixed with Lugol's iodine and observed under inverted microscope to assess the embryo-larval development and to calculate the Integrate Toxicity Index (ITI). Stable isotope analysis was conducted both on the experimental diets and on eggs produced at the beginning of the experiment (Egg-W), and at the end of the feeding trial (Egg-A, Egg-F, Egg-K, Egg-M, Egg-D).

Results

According to SIA, eggs produced by sea urchins fed with the experimental formulations diverged from $\delta^{13}C$ and $\delta^{15}N$ initial value towards the respective diets (Figure 1). ITI showed a different performance in larval development for the five experimental diets (Figure 2), with Diet-A presenting the best results thanks to the highest percentage of normorformated plutei, followed by Diet-M and Diet-D characterized by high amount of early normoformed plutei. Differently, Diet-F and Diet-K presented high relative abundance of abnormal pluteus, resulting in higher ITI values.

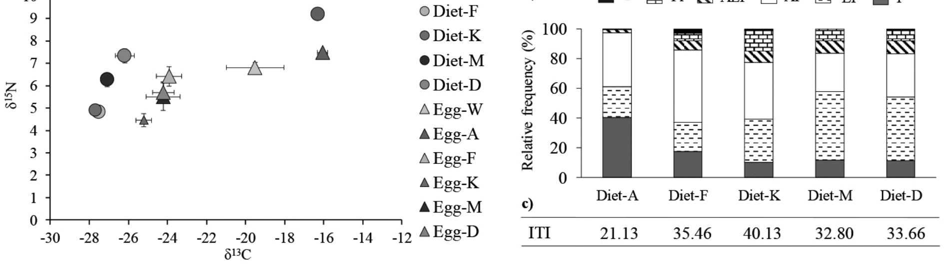

Figure 1. (a) Isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of the five diets and of the eggs produced before the start of the feeding experiment (Egg-W) and by sea urchins fed the respective diets. (b) Relative frequency malformation observed in larvae produced by sea urchin fed with the five experimental diets and the respective integrate toxicity index (ITI) value. Normal pluteus (P), early pluteus (EP), abnormal pluteus (AP), early abnormal pluteus (EAP), prism (Pr), gastrula (G).

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Discussion and Conclusion

Diet effect on produced eggs was clearly highlighted by changes in isotopic values, with Egg-A that showed an increase in both $\delta^{13}C$ and $\delta^{15}N$, according to Diet-A isotopic signature, while egg produced by sea urchin fed the four lettuce-based diets were characterized by a more negative $\delta^{13}C$ and a lower $\delta^{15}N$, resembling isotopic values of the respective diets and confirming diet assimilation [5]leading to improved productivity, sustainability and further diversification. In the case of aquaculture nutrition, a vast amount of knowledge has accumulated in relation to different physiological responses elicited by experimental diets and feeding regimes. By measuring assimilation, inferences can be made about the specific dietary components that were digested, incorporated into tissue and used for metabolic functions. One of the most common methods applied to estimate assimilation consists of measuring stable isotope values at natural abundance levels in feeding items and consuming animals. Isotopic measurements have been of great assistance to identify nutrient sources contributing to the growth of larval and juvenile organisms. The techniques have also been useful to determine nutrient flows in several types of aquaculture systems. The present manuscript reviews the most recent applications and findings derived from studies that have used stable isotope analyses to (1. Analysis on larval development confirmed that broodstock diet could deeply influence reproductive success affecting larval development [6]embryos and early larvae of the tropical echinoid Tripneustes gratilla (Linnaeus 1758. The higher relative amount of normal plutei observed for Diet-A suggest a faster development and thus better performance in larval production. Present findings are in contrast with De Jong-Westman et al. (1995) that found the worst performance in larval development with sea urchin fed kelp rather than in sea urchin fed formulated feed, due to the low quality of broodstock diet. Nevertheless, the high production of malformed or delayed larvae produced by sea urchins fed with the vegetable base diets enriched with animal meal, usually considered high nutritional source for sea urchins, confirm that also nutrient quality could affect larval development, as observed by other authors [8]"ISSN":"00448486","abstract":"This study investigated the growth and fatty acid composition of the sea urchin larvae Paracentrotus lividus fed on four types of diets: the microalgae Dunaliella tertiolecta, two types of microencapsulated formulated feeds and a concentrated algal paste. The larvae were successfully raised to metamorphosis on three diets, including the microalgae and the two formulated feeds. No significant difference was found in survival rate and metamorphosis rate for larvae fed with microalgae, formulated feeds, or microalgae plus formulated feeds. The fastest growth rate was obtained for larvae fed with microalgae, while normal growth and survival were only achieved for larvae fed with formulated feeds at a high frequency feeding rate. Normal larval development was not supported by either of the formulated feeds at a low feeding rate. A concentrated algal paste was also used for the low ration experiment, but failed to support any larval growth. Post-larval development was better for larvae fed on the formulated feeds, presumably reflecting the nutritional value of these feeds. P. lividus larvae grew well on D. tertiolecta which had a relatively low content of protein (37% dry weight. The best performance of diet A suggests that macroalgae contain macro- or micronutrients in an adequate proportion to promote larval development. Therefore, the introduction of an aliquot of macroalgae in the broodstock diet should be considered in echioculture.

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MACHINE LEARNING FOR OBJECTIVE ESTIMATION OF FISH LOSSES CAUSED BY CORMORANT AND OTTER

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Introduction

The Eurasian otter (*Lutra lutra* L.) and Great cormorants (*Phalacrocorax carbo sinensis*) belong to the piscivorous top predators causing high losses of fish in aquaculture and natural water bodies. The otter is a protected species both in many countries and by the EU law or international conventions. Its targeted hunting is prohibited in the Czech Republic and also many other countries, and therefore, the fish farmers are getting financial compensation based on the estimation of otter activity in their farming facilities. The appropriate agency of nature protection regularly estimates the number of otters based on spraints counting or footprints occurrence in five-year periods. These methods of monitoring are, however, time-consuming and must be done by a trained person. This kind of monitoring is an indirect method without any information about otter behavior around the ponds, and it is not supported by any records of the otter itself. The monitoring (direct counting) cormorant on Central-European carp (*Cyprinus carpio*) ponds is currently the only possible source of information for the evaluation of the extent of losses they cause to fish farmers. This way of monitoring is based on the regular (optimally daily) counting performed by the farmers as the applicants for compensation; however, it can be easily biased by irregular observations and mistakes in the counting of individual birds, particularly when cormorant flocks consist of tens or even hundreds of individuals. Such monitoring is not supported by any kind of records except for the observer's notes on counting and can therefore be considered subjective to some extent. We introduce a new objective methodology of otter and cormorant documentation which eliminates the biases and mistakes of human monitoring, automatizes the detection, provides a record for further evaluation, and documents the otter and cormorant behavior in a particular pond area. The methodology is based on the neural network detection of the otter in the video records from the photo traps, which monitor the selected sites. The cormorants are detected and counted in the images of a thermal camera mounted on the UAV. The solution and web services system were implemented for automatic detection of the otter and cormorant. It provides statistical information about predators' appearance at the pond and records their movements. The methodology can be adapted to any animal species which can be monitored by the photo traps or AUV.

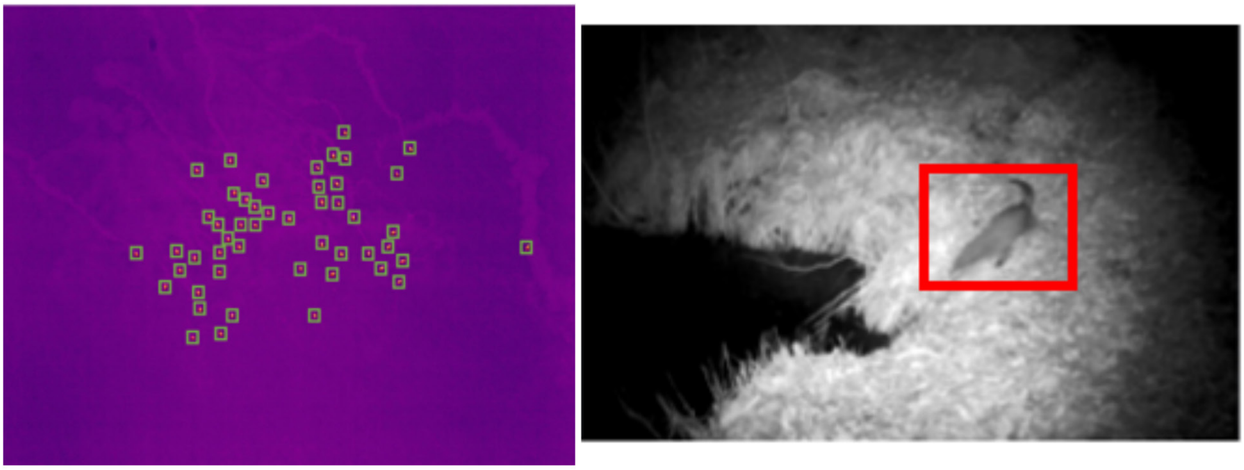

Figure 1. Left – thermal image of cormorants sitting on the water surface. Green rectangles depicts automatically detected cormorants. Right – image from the photo trap video sequence containing the otter. Red rectangle depicts automatically detected otter.

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Materials and methods

The data collection was performed for three years (2019 – 2021) at two carp pond farming sites in South Bohemia (Czech Republic) - pond system area nearby České Budějovice (hereafter CB, 49.0194933N, 14.3553972E) and the pond system nearby Nové Hrady (hereafter NH, 48.7986167N, 14.8102142E). The CB pond system area consists of 16 ponds (40 ha – 520 ha) used as ongrowing and overwintering carp (*Cyprinus carpio*) pond facilities. The NH pond system consists of 10 ponds (0.2 – 3.5 ha) mainly used as a nursing and/or ongrowing carp ponds. The ponds are arranged in a system mutually interconnected by canals. The presence of otters and cormorants was confirmed in both sites by direct monitoring of spraints, typical pathways, and observations. The monitoring of otter spainting was performed on three occasions (March 2020, February, and March 2021) at DM and five times at NH pond system (November 2019, March and May 2020, January and May 2021). The monitoring confirmed the presence of at least three otter individuals at NH pond system and at least five individuals at the DM pond.

The dataset for cormorant monitoring consisted of images from the thermal camera from 15 ponds for two years (2020 – 2021). The spring and autumn period of cormorant migrations has been documented by the datasets. More than 500 images from UAV were recorded. The data comprise 3 to 450 cormorants in one image under different weather conditions (sun reflectance, snow, rain). In addition to cormorants, three other bird taxa (gull, duck, goose) were recorded on the images.

The dataset for otter monitoring consisted of the video records from 10 ponds (20 cameras) for three years (2019 – 2021) from two locations. The video records covered the year's seasons with different weather conditions (sunny, snow, fog). More than 800 video records documented the otter (1-6 individuals) presence, and more than 14000 video records captured other animals or detections caused by the weather conditions (sun reflectance, snow, rain).

DJI M300 drone with H20T thermal camera was used for cormorant imaging. The thermal camera H20T has a resolution of 640*512 pixels and a spectral band 8-14 µm. It is also equipped with a color zoom camera with 5184 × 3888 pixels resolution. The images were taken from the top (the image plane is parallel to the water surface) at an altitude of 80 meters to eliminate overlapping of individual birds as may happen to the human observer from the viewpoint on the pond bank.

Four types of photo traps (Bunaty full HD, Bunatty wide full HD, UOVision LTE GLORY, Evolveo StrongVision 4GA) were used for data collection of otters to compare the differences in the accuracy of otter video recording. All photo traps are equipped with a passive infrared sensor (PIR) for object detection. A near-infrared illuminator at the wavelength of 940nm was used for night video recording in all photo traps. The optimal position of the photo traps was tested during the three years of monitoring.

The automatic detection of otters in the video records was implemented as a combination of motion detection followed by convolutional neural network classification into two classes: otter / no-otter. The automatic detection of cormorants in the thermal image was implemented as detection of bright spots in the image. The temperature of cormorants is significantly higher than the temperature of the background.

Results and discussion

The accuracy of otter detection was 86%, and the accuracy of detected otter classification was 98%. The accuracy of detection of cormorants was 96% in the thermal images. Both methods were implemented in the form of web service to be easily available for fish farmers and the bodies involved in fish loss estimation. The web interface is available here: http://wat.frov.jcu.cz/ login: uks.demo@email.cz, password: Slun94koSv9t9. The tools enable the fish farmers to simplify and objectify the process of fish loss estimation. The system also provides evidence of the otter and cormorant activity in the monitored region.

Acknowledgments

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BETANODAVIRUS VARIANTS CIRCULATING IN THE MEDITERRANEAN: A NOVEL, QUICK AND AFFORDABLE METHOD TO IDENTIFY GENOTYPES AND REASSORTANT STRAINS

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Introduction

Viral nervous necrosis (VNN) otherwise known as viral encephalopathy and retinopathy (VER), is the most threatening infectious disease in Mediterranean aquaculture. VNN is caused by the nervous necrosis virus (NNV) a bisegmented ssRNA+ virus included in the genus *Betanodavirus*, family *Nodaviridae*. NNV genome consists of two molecules named RNA1 and RNA2, which encodes for the RNA-dependent RNA polymerase and the coat protein, respectively. Four genotypes of NNV have been so far described: RGNNV, SJNNV, BFNNV, TPNNV (Sahul Hameed et al., 2019). Furthermore, reassortant strains have emerged from the reassortment between the RGNNV and the SJNNV genotypes and named RGNNV/SJNNV and SJNNV/RGNNV according to the RNA1 and RNA2 origin. Currently, several NNV strains are co-circulating in the Mediterranean Basin with a high prevalence of the RGNNV genotype and the RGNNV/SJNNV reassortant strain and a more limited diffusion of the SJNNV genotype and the SJNNV/RGNNV reassortant (Bandin and Souto, 2020; Volpe et al., 2020). So far, identification of NNV genotype and reassortant strains is based on amplification, sequencing and phylogenetic analysis of both viral genome RNA molecules requiring time and expertise. As viral identification is an essential information for the management of the disease in the field (Toffan et al., 2021), the development of an easy and affordable method to genotype betanodaviruses was developed in the framework of the H2020 Peformfish Project, an industry-driven project targeting industry defined priorities.

Materials and methods

RGNNV-RNA1, RGNNV-RNA2 and SJNNV-RNA2 specific primers were designed based on conserved regions within each genotype's sequences. The primer sets were selected according to their position within each genotype's RNA sequence, the melting temperature and the predicted amplicon size to amplify fragments of different sizes specific for RGNNV-RNA1, RGNNV-RNA2 and SJNNV-RNA2. After optimisation the novel multiplex RT-PCR assay was tested for analytical specificity and sensitivity and for repeatability thought intra- and inter-assay variability tests. Furthermore, the diagnostic sensitivity and specificity was calculated using seventy-six samples including 50 European seabass and 23 gilthead sea bream brains and 3 Artemia salina (nauplii) samples and comparing the mRT-PCR to the OIE validated real-time RT-PCR taken as the "Gold Standard". The robustness of the method was assessed testing samples included in the third VER interlaboratory proficiency test (VER-IPT) organised by the OIE reference laboratory. Finally, the accuracy of the genotyping performed via the new developed mRT-PCR was assessed subjecting to the conventional genotyping a selection of positive samples which reported different range of results at the mRT-PCR assay.

Results

A one-step multiplex RT-PCR was developed to detect and simultaneously identify the presence of RGNNV, SJNNV or one of their reassortant strains through the amplification of a specific band pattern for each genotype/reassortant strain. No cross-reactivity with viruses and bacteria frequently associated with gilthead seabream, European seabass and marine environment was observed. The mRT-PCR showed a sensitivity ranging from $10^{2.3}$ to $10^{3.4}$ $TCID_{50}$ ml^{-1} depending on the viral strain and a high repeatability with negative, weakly, and distinct positive samples. The mRT-PCR accurately detected no NNV-RNA in NNV-negative samples and produced at least one specific amplification band in all NNV-positive samples assuming a diagnostic specificity and sensitivity of 100%. The application of the mRT-PCR to the samples from the third VER-IPT enabled to correctly detect their positivity/negativity and to genotype them. Multiplex RT-PCR was able to assign to a genotype the RNA1 of all the positive samples and the RNA2 of 88% of positive samples.

(Continued on next page)

Discussion and Conclusion

Several NNV strains are currently co-circulating in the Mediterranean Basin. Genotyping and reassortant identification are an essential information for the management of the disease in the field. However, proficiency tests performed by the OIE reference laboratory pointed out a limited capability to properly identify the NNV variants (Toffan et al., 2021). Identifying the genotypes and reassortant strains is so far based on traditional genotyping that consists in amplifying and sequencing of both viral genome RNA molecules. Only the analysis of both viral genome segments (RNA1 and RNA2) led to reassortant identification. This method is time-consuming and requires highly specialised staff and equipment, resulting in a limited number of laboratories able to perform a complete and correct viral species identification. The developed multiplex RT-PCR represents an easy, rapid, and affordable method to identify NNV variants circulating in the Mediterranean: RGNNV, SJNNV and the reassortant RGNNV/SJNNV and SJNNV/RGNNV strains. The method can find wide application in several sectors of the study of the VNN contributing to improve its knowledge and therefore leading to a better disease control. It can also be used for confirmatory diagnosis in NNV outbreaks in the Mediterranean increasing laboratories' capacity to correctly identify the betanodavirus genotype.

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MUSSEL MEAL AS A POTENTIAL INGREDIENT IN DIETS FOR THE WHITELEG SHRIMP (*Litopenaeus vannamei)*

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Introduction

Litopenaeus vannamei, commonly known as Pacific white shrimp or whiteleg shrimp, is the most cultivated shrimp species worldwide with a total volume of more than 6.5 million tons in 2018 and had a growth in production of 8.78% in 2020 (FAO, 2020). *L. vannamei* is a tropical species, originally farmed in tropical areas and known to be sensitive to low temperatures. Due to its high economic value, the farming areas have been expanded to the sub-tropical areas. Therefore, low temperature has become one of the major constraining factors in the *L. vannamei* production. To overcome temperature related environmental stress factors, the robustness of the *L. vannamei* must be improved.

Material and methods

Five diets based on the formulation of a commercial shrimp diet were tested with different inclusion levels of mussel meal (*P. perna*): 0% (control), 1%, 2%, 3% and 4%. The experiment was carried out at the Marine Shrimp Laboratory (Laboratório de Camarões Marinhos – LCM/UFSC) at Barra da Lagoa, Brazil, where in total twenty polyethylene tanks of 400 liter were used (n = 4). Each tank was stocked with 40 shrimps (3.5 ± 0.5 g) and were kept for 8 weeks at a temperature of 28 ± 1 °C under constant aeration with a 100% daily exchange of water.. During these 8 weeks growth was measured for the different treatments and after the 8 weeks a potential lethal cold shock treatment was performed to verify the robustness of the shrimps.

Results

The final weight (g) and weekly weight gain (g/week) were significantly higher in the 1% and 2% mussel meal diets after 8 weeks (figure 1 & figure 2). The survival was >94% after 8 weeks in all treatments.

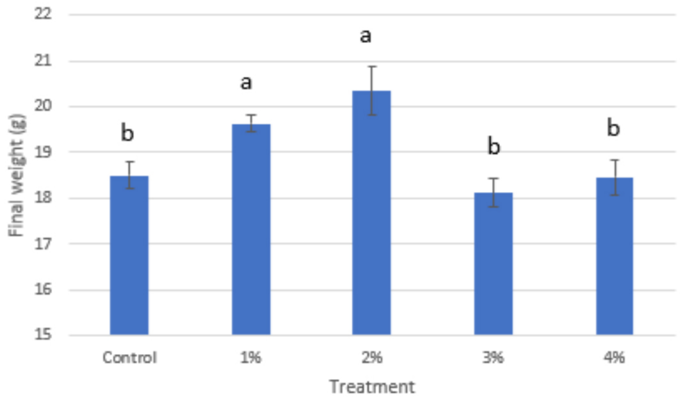

Fig 1. Final weight in grams from the five treatments after 8 weeks. The final weight from the 1% and 2% mussel meal diets were significantly higher than the Control, 3% and 4% of mussel meal diets.

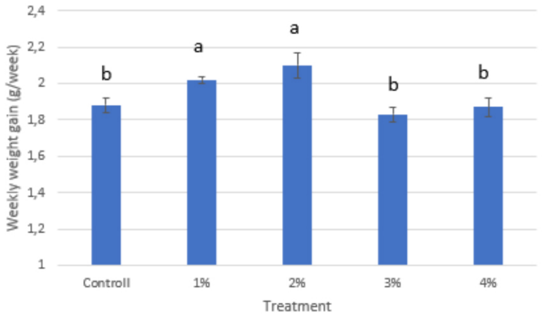

Fig 2. Average weekly weight gain in grams from the five treatments in 8 weeks. The weekly weight gain from the 1% and 2% mussel meal diets were significantly higher than the Control, 3% and 4% of mussel meal diets.

Conclusion

Mussel meal can be used as a potential ingredient in diets for the whiteleg shrimp *(Litopenaeus vannamei)*. It even improved growth when putting 1% of 2% of mussel meal in the diets.

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BACTERIAL COMMUNITY IN AFRICAN CATFISH (*Clarias gariepinus*) RECIRCULATION AQUACULTURE SYSTEM UNDER DIFFERENT STOCKING DENSITIES

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Introduction

African catfish (*Clarias gariepinus*, Burchell 1822) is a good candidate for Recirculating Aquaculture Systems (RAS), since it tolerates adverse environmental changes (FAO, 2020). To maintain optimal water quality relies on certain physicochemical parameters (Keramat Amirkolaie, 2008) and on the microbial community (Lucas and Southgate, 2012). However, the identity and activity of nutrient cycling bacteria population remains poorly understood while producing *C. gariepinus*. The aim of the present study is to describe the microorganisms community composition (at phylum, family and genera taxonomical levels) during *C. gariepinus* rearing trial, testing three different stocking densities: intensive (S1), semi-intensive (S2) and extensive (S3).

Material and methodology

Samples of water were collected in triplicate from three RAS at the research facility "FishGlassHouse", University of Rostock (Germany). Three sampling groups were considered: i) filtered water that enters the fish tanks (water inflow); ii) water leaving the fish tanks (water outflow), and iii) water from the sedimentation tank (sedimenter water). Each system had specimens of *C. gariepinus* stocked at three different densities: extensive (25 individuals/m^3), semi-intensive (70 ind./m^3) and intensive (120-130 ind./m^3). Water temperature, dissolved oxygen (%), pH, salinity (‰), conductivity (μS cm^{-1}) and redox potential (mV) were measured. Molecular approaches (RT-PCR and Miseq Ilumina sequencing) targeting the V3-V4 16s rRNA hypervariable region were used to construct a library of amplicon sequence variants (ASV) and assess microbes' identification, abundance and diversity. Statistical analyses were performed at phylum, family and genus levels using different packages in R version 4.1, and custom R scripts.

Results

Overall, Proteobacteria and Bacteroidetes were the predominant phyla in all types of samples. The S1 and S3 groups showed different patterns, as taxonomic abundances of certain dominant phyla tended to change between in- and outflow water. The S2 group had the highest accumulative abundance of ASVs and tended to be similar between sample groups. All samples from the S2 were grouped with less disparate separation between them, contrarily to S1 and S3, where few families appear to be dominant. The following genus of pathogenic bacteria were identified at all systems: *Mycobacterium*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Yersinia*, *Rickettsia* and *Aeromonas*.

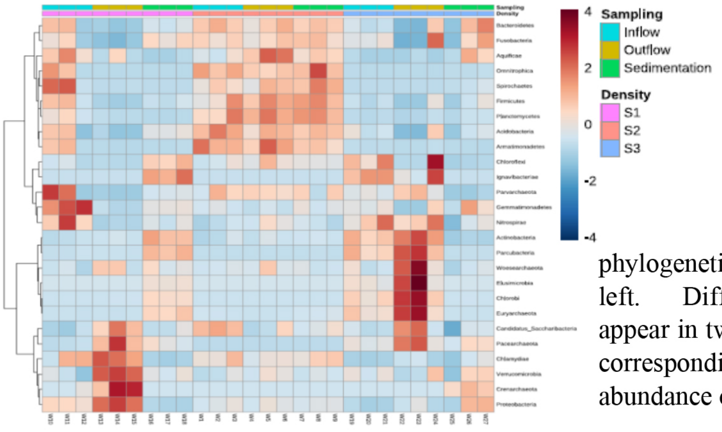

Fig. 1. Heatmap showing the relative abundances of the 26 most abundant families from water inflow, outflow and sedimenter at S1, S2 and S3. The phylogenetic tree appears on the left. Differential abundances appear in two colors, red and blue, corresponding to low and high abundance of ASVs respectively.

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Discussion

Our study comparing systems with different stocking densities is a clear example of community structural variability influenced by fish biomass. In this study we detected 42 phyla at three sample points of the RAS. The most abundant groups identified at all compartments of S2 included sulfur redactor families. Other abundant groups were mainly heterotrophic. When high amounts of organic carbon are available, heterotrophic bacteria may outcompete nitrifies for available oxygen and space, and the nitrification rate decreases (Michaud et al., 2006). In general, at S1 and S3 we detected high diversity of metabolic functions, and the dominant families detected at water inflow and outflow participate at the nitrogen cycle and organic matter degradation. Heterogeneity of communities is usually attributed to environmental changes (Krotman Y, 2020) or water treatment processes (Ma X, 2017). Potential pathogens were more abundant S2, which had low water exchange and high fish biomass, although it may not directly affect fish health and/or growing performance. Each RAS holds dynamic microbial communities, which change in response to the accumulation of nutrients, and participate to the maintenance of proper water quality (Califano et al, 2017). This knowledge can be used to further develop *C. gariepinus* RAS, detecting potential shifts of the microorganism's populations with increasing fish biomass and possible implications on the fish health.

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INTESTINAL MICROBIOTA CHARACTERIZATION IN *Salmo Salar* WITH CLINICAL SIGNS OF *Piscirickettsia Salmonis* IN CHILEAN SALMON FARMING

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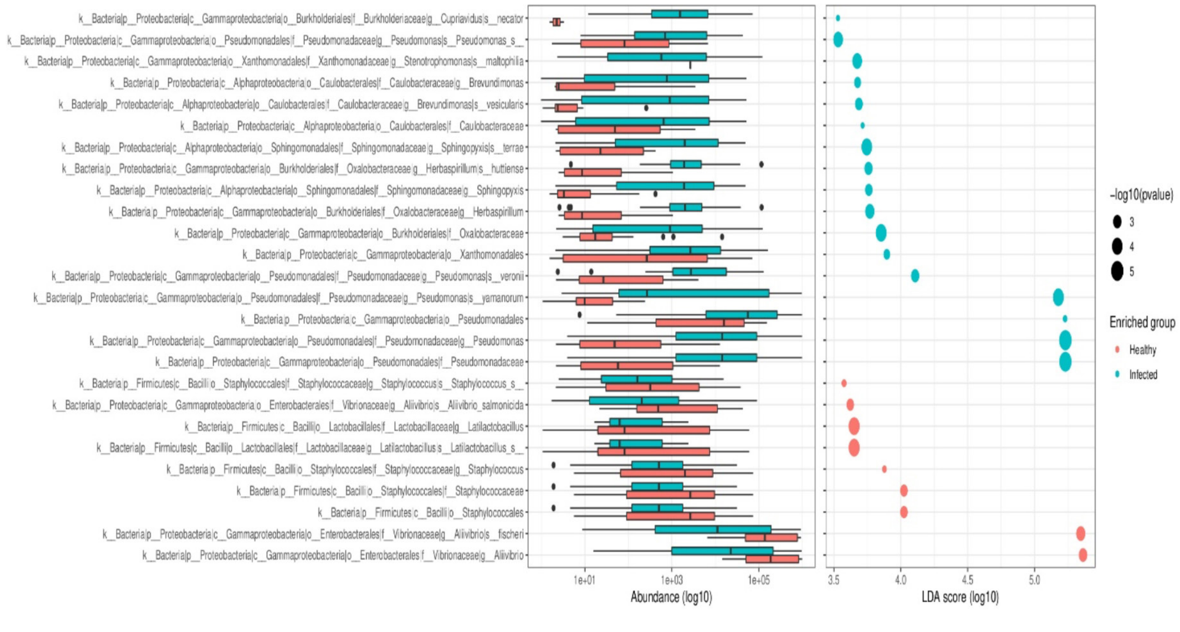
Introduction

In Chile, SERNAPESCA (the National Fisheries Service) has identified *Piscirickettsia salmonis* (SRS; Salmonid Rickettsial Septicemia infection) as the most severe health problem facing the Chilean salmon industry owing to its highly aggressive nature, recurrent outbreaks, and widespread transmission, among other cultivated salmonid species. The total mortality of salmon due to specific diseases in Chile is associated with SRS and is in the order of 50% to 97%, which translates into direct and indirect annual losses of close to 700 million dollars. The changes caused by the pathogen *Piscirickettsia salmonis* in the gastrointestinal microbiota in *Salmo salar* during infectious outbreaks due to this pathogen in Chile are unknown and not widely researched. Therefore, knowing which microbial species in the gut are affected or favored by the pathogen *Piscirickettsia salmonis* is the main objective of this research.

Material and Methods

The salmonids analyzed in this study correspond to the Atlantic salmon (*Salmo salar*) (weight: 2869.34 g +/- 3160.42 g) from six Chilean farms located in the Los Lagos and the Aysén Region. Salmonid samples were taken during infectious outbreaks with *Piscirickettsia salmonis* in four salmon farming: C1, C2, C3, and C4. During January and February, summer in the southern hemisphere. Samples were taken from six *Salmo salar* per farm, with clinical skin signs of infection by *Piscirickettsia salmonis* in each salmon farm, as mentioned earlier. Additionally, 12 healthy fish from two salmon farms (C5 and C6) that did not present reports of infectious outbreaks of *Piscirickettsia salmonis* were analyzed to confirm that the *Salmo salar* taken as control did not present the pathogen infections, clinical, histological analyses of the organs (kidney, liver, and spleen) were performed.



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The 16S rRNA primer pair, 515F GTGYCAGCMGCCGCGGTAA / 806R GGACTACNVGGGTWTCTAAT, evaluated the microbial ecology of each sample on NovaSeq with methods through the bTEFAP® DNA Analysis Service. Each sample was subjected to a one-step 35-cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). After PCR, all amplicon products from different samples were mixed in equal concentrations and purified using SPRI beads.

Raw reads were quality trimmed using Fastp v0.20.1 (REF), removing the first 10 nucleotides, discarding all reads with one or more Ns, then using the right to left sliding windows method with size 4 and minimum q-score 30 and finally, all reads with mean q-score lower than 30 were discarded. High-quality reads were de-noised, filtered against chimeric PCR artifacts, and merged using the R package DADA2 version 2.1.18 (REF), producing clean 16S amplicon sequence variants (ASVs). The taxonomic assignment was performed with the same R package using as reference SILVA 138.1

Results

This study showed that the main bacterial phyla of all salmon sampled are Actinobacteria, Firmicutes, and Proteobacteria. The research also found that the control groups in the study (*Salmo salar* without clinical signs of SRS infection) have concentrations of this pathogen in their gastrointestinal system. The study also found a high concentration of Proteobacteria in the gut of all *Salmo salar* sampled, suggesting that the salmonids are dysbiosis. Interestingly, this study found co-occurrence networks between the pathogen *P. salmonis* and other aquaculture pathogens that did not manifest clinical signs of infection in the sampled salmon. Finding other pathogens in cohabitation with pathogen *P. salmonis* is undoubtedly one of the most exciting findings of this research.

SUSTAINABLE BUSINESS MODEL INNOVATION IN A LOW CARBON OFFSHORE AQUACULTURE VALUE CHAIN: ORGANIZATIONAL FACTORS AND INSIGHTS FOR MANAGERS

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Introduction

This paper examines how firms engage in sustainable business model innovation (SBMI) in a multi-stakeholder public-private offshore aquaculture innovation project in Norway. To address Grand Societal Challenges (GSCs), firms engaging in SBMI must also collaborate with other stakeholders, including research institutions, policymakers, NGOs, and the general public. Further, organizational design can impact firms' ability to engage in SBMI. In this paper, we explore these challenges and their implications in the case of a new green offshore aquaculture value chain in Norway. Leveraging an action research case study approach, we analyze how organizational factors impact SBMI in this context and build theoretical and managerial insights for how firms engaged in multi-stakeholder innovation projects can succeed with SBMI, particularly in the aquaculture industry.

Background

Until now, salmon aquaculture in Norway has only been in sheltered inshore waters. But as production has increased substantially along the coastline negative environmental externalities have increased. Offshore aquaculture has recently received attention as an avenue for sustainable production growth. Offshore ocean areas, representing 90% of the Norwegian economic zone area, provide opportunities for reducing environmental externalities substantially. However, offshore aquaculture faces extreme wind and wave conditions, long distances from shore to farms, and significant up-scaling of production with potential new biological and technological challenges. In order to become economically and environmentally sustainable offshore aquaculture requires an entirely new value chain with technological innovations at all stages, and multi-billion USD investments to achieve economies of scale. In 2020 the Norwegian government established the 'Green Platform' innovation program, primarily as a policy tool to meet Norway's climate gas reduction obligations under the Paris agreement. The programs aims to address Grand Societal Challenges in the green transition towards a low carbon society by stimulating the establishment of new consortiums of companies and research institutions with sufficient complementary resources for large-scale innovation projects. The case presented here examines one of the consortiums that successfully applied for Green Platform co-funding from government: a coalition of 7 aquaculture value chain companies and 10 research institutions, working to develop a low emission offshore aquaculture value chain. The first and second authors were involved in preparing the application for this project. The project involves research and innovations on electrification of transportation and farming processes, new salmon feeds, and digitalization and automation of farming operations, with a. total budget of 185 million NOK (approx. 18 million EUR), including government co-funding of 93 million NOK.

Methods

We leverage a qualitative action research case study approach (Bocken et al., 2017; McManners, 2016). This approach "combines the focus on inquiry and action offered by action research with the case study methodology described by Yin (2013). It allows researchers to go beyond the role of neutral observer to a participatory role whilst retaining academic rigour (McManners, 2016), and is seen as instrumental in the transition to a sustainable world (Gustavsen, 2008; McManners, 2015)" (Bocken et al., 2017, p.10). We followed Yin (2013) in selecting this case for a single case study, as it is particularly unique and offers unusual revelatory potential. Inductive methods are increasingly recognized in the management literature as being appropriate for addressing Grand Societal Challenges (Eisenhardt et al., 2016). Interview questions were developed and data was analyzed following a grounded theory approach (Gioia et al., 2013; Locke, 2001). Data included semi-structured interviews (11), participant observation (30+ meetings), and document analysis. Interview data was categorized into 1st- and 2nd-order concepts and themes, and triangulated with observation and textual data.

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Results

Preliminary results reveal themes which provide insight for SBMI in collaborative contexts. Level of vertical integration, organizational culture, existing infrastructure for open innovation, public innovation requirements, and project context impact SBMI. Action research can influence project outcomes and lead to sustainability improvements. Firms engaging in collaborative SBMI in collaborative contexts can lead to broader sustainable ecosystem innovation (Snihur & Bocken, 2022).

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POSITIVE WELFARE EFFECTS OF PHYSICAL ENRICHMENTS FROM THE NATURE-, FUNCTIONS- AND FEELING- BASED APPROACHES IN FARMED RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

In captive animals, the concept of positive welfare is defined as the physical and mental states that exceed what is strictly necessary for short-term survival (Fife-Cook and Franks, 2019). Therefore, it becomes essential no only to prevent poor welfare, but also to give animals greater opportunities to experience positive affects by generating various forms of comfort, pleasure, stimulation, interest, and sense of safety in order to induce long-lasting positive affective states, close to the positive welfare concept. Environmental enrichment is a deliberate increase in environmental complexity and is one of the strategies investigated to improve the living conditions of animals reared in stimuli-deprived environments, including farmed fish. Physical enrichment, a category of environmental enrichment, consists in adding structures (stones, plants, kelps, sand, gravels) into the rearing tanks, but is rarely used in aquaculture. Fish welfare can be approached from three different angles: nature-, functions- and feelings-based (Huntingford et al., 2006). Physical enrichment is closer to the nature-based approach meaning that fish should live in an environment close to the natural habitat of the species, in order to promote more natural behavior (Martins et al., 2012). According to the functions-based welfare approach, fish must be able to maintain their biological functions and zootechnical performances. Growth (Kientz et al., 2018) and health (Karvonen et al., 2016) were shown to be improved when adding physical structures into the tanks of salmonids species. The third approach to consider fish welfare is “feeling-based” and set in terms of subjective mental states. From this point of view, negative emotional experiences must be decreased and positive experiences should be promoted. Providing fish with physical enrichment may be an effective way to meet the positive welfare concept from the feeling-based approach also by stimulating fish, encouraging exploration and facilitating their curiosity and interest.

In the literature investigating the influence of enrichment on fish welfare, the three different perspectives are often disconnected and rarely evaluated within a whole experiment. The present study aimed to investigate whether physical enrichment had positive effects on the welfare of rainbow trout, the first continental fish species produced in Europe, through these nature-, functions- and feeling-based approaches.

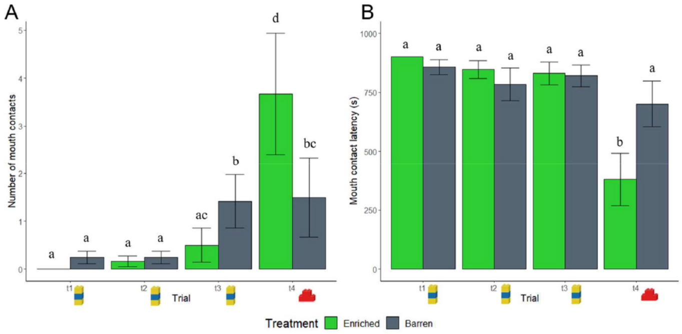

Fig. 1. Number of mouth contacts with objects (A) and mouth contact latency (B) after the introduction of the objects at each trial (t1, t2, t3, t4: novel object) of trout raised in enriched environment (E: green) or barren environment (B: grey) during the novel object test. Values are means and the mean standard error is represented. Statistical differences are represented by different letters (P<0.05, n=12).

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Material and Methods

Female triploids rainbow trout (*Oncorhynchus mykiss*) were split in two experimental treatments: an enriched environment (E) and a barren environment (B) from 99 days post-fertilization (dpf) to 333 dpf. The enriched environment was composed by PVC pipes, plastic plants and white stones. For the nature-based approach, groups of rainbow trout (3 tanks/treatment; 30 individuals each) were observed in their home tanks (enriched vs. barren) provided with video cameras for 4 weeks (from 162 to 187 dpf) to analyze fish group behavior (dispersion, aggression and stereotypies). Fin erosion was also recorded at the end of this period as a marker of aggression. To assess welfare from the functions-based approach, fish growth was monitored (from 102 to 333 dpf) and immune analyses (lysozyme, complement component ACH50) were carried out at 208 dpf. Two behavioral tests were performed to assess fish welfare from the feeling-based perspective: (i) a novel-tank test to evaluate the fear-related responses (behavior and plasma cortisol release) at 208 dpf, and (ii) a standard novel object paradigm to characterize fish boldness and curiosity (at 250-260 dpf). The analysis of the behaviors expressed during these tests was performed with the EthoVision XT software.

Results and discussion

Our findings demonstrate that providing physical structures in the environment of farmed rainbow trout decreases aggressive behaviors in the group, as confirmed by lower fin erosions. Physical enrichments might restrict territorial range and visual contact, thereby decreasing the probability of encountering and consequently reducing aggression towards conspecifics (Zhang et al., 2021). Moreover, the possibility for subordinate fish to hide into shelters is likely to promote positive welfare for those fish. Decreased aggressive behaviors, accompanied by fewer stereotypic behaviors, induced more opportunities for resting and exploring in shoal cohesion close to the structures. Heightened shoal cohesion is considered as a marker of positive emotions in zebrafish (Franks et al., 2018). The lower level of aggression probably contributed to improve growth, without any effect on health parameters. When subjected to the emotional reactivity test, enriched fish displayed fewer fear-related behavior (decreased angular velocity and higher exploration) but plasma cortisol levels remained similar between groups. When exposed to a novel object, enriched fish were bolder and less neophobic, spending more time close to the object (Fig. 1), without exhibiting anxiety-like behaviors. Enriched environment seems to give captive fish opportunities to experience positive affects by generating various forms of stimulation, interest, and sense of safety, inducing long-lasting positive affective states, as observed by lower anxiety-related behaviors, higher exploration, and decreased neophobia towards novelty, meeting the positive welfare concept.

Conclusion

Complexifying the environment through the addition of physical structures which stimulate and encourage fish to explore promotes rainbow trout's welfare in farming conditions, according to the nature-, functions- and feeling-based welfare approaches.

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DETECTION OF VIRULENCE FACTORS IN *L. garvieae* BELONGING TO SMALL-SCALE TROUT PRODUCTIONS IN THE MEDITERRANEAN AREA

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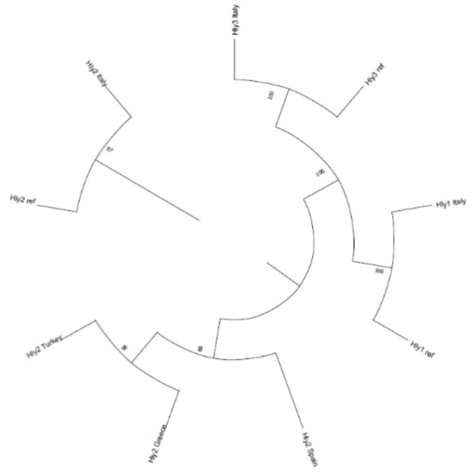
Introduction

Aquaculture production has expanded significantly in recent years. Nonetheless, infectious diseases remain a key concern, preventing future escalation. *L. garvieae,* a warm-water pathogen, is one of the main issues for aquaculture in the Mediterranean area. It is the aetiological agent of lactococcosis in freshwater and marine farmed fish, a disease marked by hyperacute hemorrhagic septicemic infections. The Mediterranean's moderate climate and higher water temperatures, due to the global warming, contributed to the emergence of lactococcosis outbreaks in this region. Virulence factors seem to act as main actors of *L. garvieae* pathogenicity. One of these factors is the capsule: the link between the pathogenicity of the strains and the capsule is not fully understood. However, differences in the capsule's polysaccharide composition might explain the virulence or non-virulence of some strains. Other factors are hemolysins because of their lytic actions. Hemolysins and their role in virulence and disease have been examined at the molecular level in various pathogenic bacteria, including *L. garvieae*. By generating pores and altering phospholipid structures in the membrane, their enzymatic actions promote the lysis of red blood cells and phagocytes. On blood agar, *L. garvieae* displayed hemolytic activity, and three hly genes were found in the genome: hly1, hly2, and hly3. In the context of the European project SUPERTROUT (Improving SUstainability and PERformance of aquaculture farming systems: breeding for lactococcosis resistance in rainbow TROUT), aimed at sustaining small-scale farming systems of trout production in the Mediterranean area by using an innovative approach to control lactococcosis, archivial strains collected in the Countries participating to the project (Italy, Spain, Greece and Turkey) were characterized for hemolisin genes and for the capsule cluster genes.



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Materials and Methods

80 archival strains from local rainbow trout farms (one for each Nation participating to the project) collected in the five years before this study were analyzed.

The pathogenicity of the three hemolysin genes was determined by sequencing the amplicons obtained by positive strains. DNA was extracted using the freeze-boiling thawing method and amplified as described by Teker et al. (2018). For DNA sequencing, BigDye 3.1 chemistry was used. Sequences were aligned with the reference sequences genes present in Genbank, hemolysin 1 (GenBank accession number: MG999526), hemolysin 2 (MH316613) and hemolysin 3 (MH316614) using Lasergene (DNASTAR) software.

The presence of the capsule gene cluster in *L. garvieae* strains was screened using the protocol reported by Ture and Altinok (2016). Amplicons were confirmed by agarose gel electrophoresis, comparing amplicon size with the positive control and a 100 bp DNA ladder.

Results

A total of 80 strains from Italy, Spain, Greece, and Turkey were tested for the presence of hly1, hly2, and hly3 genes. Only the Italian strains were positive for the amplification of hemolysin 1 and 3 genes. Amplification of hly1 was negative in four strains. Hemolysin 2 was amplified for *L. garvieae* strains in all countries and presence of the hly2 gene in Italian, Spanish, Greece and Turkish strains were 100, 100, 95 and 60%, respectively. Sequences for each hemolysin were submitted to GenBank with the following accession numbers: Italian hemolysin 1 OM663244; Italian hemolysin 2 OM810256; Turkish hemolysin 2 OM810257; Greek hemolysin 2 OM810258; Spanish hemolysin 2 OM810259; and Italian hemolysin 3 OM663245. The Maximum Parsimony method was used to estimate the evolutionary history of hemolysin gene sequences. A bootstrap (1000 replicates) consensus tree was inferred using MEGA X.

Discussion and Conclusions

Data from hemolysin phylogenesis revealed a higher similarity among strains, particularly those from Greece, Turkey, and Spain, and a lower similarity with Italian strains, suggesting the strains' geographical origins. Furthermore, when compared to hemolysin 3, Teker et al. (2018) found hly1 and 2 as key virulence factors. Turkish strains seem to be less virulent than other strains based on the percentages of amplification achieved for hemolysin 2, as well as the lack of hemolysin 1 and 3, yet substantial mortality was found in the farms where the strains were collected. Because of the reciprocal interaction between host and pathogen, other variables such as host susceptibility linked to genetic determinants and environmental characteristics such as water parameters or rearing density should be addressed in the progression of the infection.

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Lactococcus garvieae AND *Lactococcus petauri,* TWO PATHOGENS FOR THE SAME DISEASE

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Introduction

Lactococcus garvieae is a well-known fish pathogen that affects a wide range of farmed and wild marine and freshwater species. Lactococcosis, caused by *L. garvieae*, is a septicemic disease of significant clinical and economic relevance in the farmed trout industry. Affected fish exhibit erratic swimming, darkening of the skin, lethargy, exophthalmia, anorexia, coelomic distention, and hemorrhages at the fin bases, mouth cavity, and operculum. Internally, affected fish have congested and enlarged kidneys, livers, and spleens, as well as hemorrhages in the brain, swim bladder, and intestine. *Lactococcus garvieae* has long been thought to be the unique *cause* of lactococcosis, but *Lactococcus petauri*, another species of the genus Lactococcus first isolated from a facial abscess in a sugar glider (*Petaurus breviceps*) (Goodman et al. 2017), has recently been linked to lactococcosis outbreaks in Europe and North America, with mortality rates of 40% (Savvidis et al., 2007; Shahin et al., 2021). The genome sequences of *L. petauri* and *L. garvieae* are quite similar, as are their morphological characteristics and biochemical profiles (Goodman et al., 2017). Differentiating *L. garvieae* and *L. petauri* isolates in diagnostic laboratories is a tough task because the molecular diagnostic methods currently available do not differentiate between both species. In the context of the SUPERTROUT European Project (https://mel.cgiar.org/projects/supertrout), we used the genomes of a collection of *L. garvieae* isolates from different sources to calculate their average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values, which are widely used for differentiating species (Chun et al., 2018), to accurately establish their correct species assignment.

Material and Methods

dDDH and ANI values calculation

The assembled genomes of 21 field Spanish isolates previously identified as *L. garvieae* and 28 genomes of *L. gavieae* available in Genbank (https://www.ncbi.nlm.nih.gov/genome/browse/#!/prokaryotes/699/) were used for ANI calculation (https://www.ezbiocloud.net/tools/ani). ANI values were computed for pairs between these genomes and the genomes of the type strains of *L. garvieae* CCUG 32208^T (VXKD01000001) and *L. petauri* 159469^T (MUIZ01000010). The pairwise ANI values use the Orthologous ANI algorithm using the USEARCH program (Yoon et al. 2017). Moreover, dDDH analysis was performed using the GGDC 3.0 method, which was recommended for draft genomes (https://ggdc.dsmz.de/ggdc.php).

Phenotypic studies

Three representative strains [*L. garvieae* CCUG 32208^T, *L. petauri* 159469T and the Spanish clinical fish isolate *L. petauri* 8831 (initially identified as *L. garvieae*)] were biochemically characterized using the Rapid ID 32 Strep, API 50 CH and API ZYM systems. In addition, the morphological characteristics of the colonies of these three strains as well as other field Spanish isolates were compared after their growth on trypticase soy agar (TSA) supplemented with 5% sheep blood and M17 agar.

Results and Discussion

Twenty-one of the 49 isolates analyzed had dDDH values ranging from 80.9 to 99.9% and ANI values ranging from 98.1 to 100% with the type strain of *L. garvieae*, indicating their accurate identification. In contrast, 19 previously identified *L. garvieae* isolates had dDDH values ranging from 80.3 to 90.8% and ANI values ranging from 97.9–99.0% with the type strain of *L. petauri*, indicating that they should be reclassified. The dDDH and ANI values of nine isolates were lower than the recommended species demarcation limits (70% for dDDH and 95–95% for ANI), indicating that they do not belong to either species. Strains isolated from various sources (fish, water, mammals, and humans) and countries were properly

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recognized as *L. garvieae*, those that should be reclassified as *L. petauri*, and those that are neither species. These findings suggest that many of the *L. garvieae* isolated from cases of trout lactococcosis and other sources should be reclassified as *L. petauri*. As a result, both species should be regarded as the causative agents of fish lactococcosis. Many *L. garvieae* isolates are identified using 16S rRNA gene sequencing or PCRs targeting this gene, and the high similarity in the 16S rRNA gene sequences of both species (99.93%) may explain why many strains of *L. petauri* have been misidentified as *L. garvieae*. It should be noted that colonies of those field isolates that should be reclassified as *L. garvieae* based on dDDH and ANI values did not have the orange-colored morphology of *L. petauri* when grown anaerobically for 24 hours at 30°C on M17 agar, nor did they have the beta-hemolytic activity of *L. petauri* on blood agar. Biochemically, the strains *L. garvieae* CCUG 32208T, *L. petauri* 159469T, and *L. petauri* 8831 were quite similar. However, both strains of *L. petauri* hydrolyzed hippurate and produced acid from methyl-D-glucopyranoside, sucrose, and tagatose, but the type strain of *L. garvieae* did not. These findings should be validated by testing a large number of isolates. To obtain a better understanding of the roles of both pathogens in the epidemiology of lactococcosis, an in-depth review of previous *L. garvieae* isolate identifications will be required, as well as the development of new molecular diagnostic tools that allow efficient and reliable differentiation between the two pathogens.

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PHENOTYPIC AND GENOTYPIC ANTIBIOTIC RESISTANCE OF *Lactococcus garvieae* STRAINS ISOLATED FROM RAINBOW TROUT, *Oncorhynchus mykiss*, IN THE MEDITERRANEAN REGION

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Introduction

Up to now, usage of antibiotics in aquaculture played a pivotal role in achieving major advances against bacterial infections. Still, Bacteria may gain antibiotic resistance and spread as a consequence of inappropriate and extensive use of antibiotics. The rapid emergence of antibiotic resistant bacteria endangers the efficacy of antibiotic and leads to the spread of resistance genes. In the present study, phenotypic antibiotic resistance against 12 antibiotics and the presence of 14 antibiotic resistance genes were evaluated in archival *Lactococcus garvieae* strains isolated from small-scale rainbow trout farms in Turkey, Italy, Greece, and Spain.

Material and methods

Twenty archival *L. garvieae* strains of each country were previously isolated from diseased rainbow trout (*Oncorhynchus mykiss*). Antibiotic susceptibility tests were performed by the disk diffusion method using kanamycin (K; 30 μg), ampicillin (AMP; 10 μg or 25 μg), gentamycin (CN; 10 μg or 30 μg), florphenicol (FFC; 30 μg), streptomycin (S; 10 μg), erythromycin (E; 15 μg), oxolinic acid (OA; 2 μg or 10 μg), flumequine (FLM; 30 μg), penicillin (P; 10 μg), amoxicillin (AMX; 20 μg or 25 μg), neomycin (N;30 μg), and oxytetracycline (OT; 30 μg) discs. Presence of florfenicol (*floR*), tetracycline (*tetA*, *tetB*, *tetC*, *tetG*), erythromycin (*ermA*, *ermB*, *ermC*), streptomycin (*strB*), β-lactamase ampicillin (*blaSHV*, *blaOXA*, *blaTEM*), quinolones oxalinic acid (*gnrA*), and quinolones flumequine (*gyrA*) resistance genes was tested by PCR.

Results

Overall, antimicrobial susceptibility tests indicated that 93.8% of the isolates were resistant to oxolinic acid, followed by flumequine (91.3%), streptomycin (88.8%), kanamycin (72.5%), and neomycin (66.3%). Whereas, the highest susceptibilities were seen toward gentamycin (100%), oxytetracycline (97.5%), florfenicol (75%), ampicillin (73.8%), and amoxicillin (72.5%). Among all strains (n=80), 96.3% (n=77) had at least one resistance gene and 57.5% (n=46) had 2 or more antibiotic resistance genes. Among the screened tetracycline genes, *tetC* (57.5%) was the most prevalent gene followed by *tetG* (33.8%), *ermC* (23.8%) and *tetB* (21.3%), whereas *floR* (2.5%), *ermA* (2.5%), *tetA* (3.8%), and *blaSHV* (6.3%) were among the least detected genes. Antibiotic susceptibility differences and variation in antibiotic resistance genes were recorded among Turkish, Italian, Greek, and Spanish archival strains which indicates possible genetic and phenotypic differences among strains. Prevalence of resistance to commonly used antibiotics in aquaculture and detection of resistance genes in strains may lead to prolonged treatments and to the increase *L. garvieae* related mortalities. This study provides a starting point for comprehensive monitoring of antimicrobial resistance of *L. garvieae* strains in rainbow trout aquaculture in the Mediterranean Region.

ROADMAP FOR DEVELOPING A MICRODIET FOR WHITELEG SHRIMP (*Penaeus vannamei*) POST-LARVAE

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Introduction

Shrimp aquaculture is considered one of the most profitable aquaculture industries. In order to respond to Whiteleg shrimp (*Penaeus vannamei*) production, hatcheries need to improve post-larvae (PL) rearing protocols. The early life-stage is a critical period in shrimp development, with the transition from live-feeds to inert microdiets (weaning) being severely influenced by diet quality. Problems related to microdiet quality have a large impact on shrimp performance in the long-term, affecting the downstream production of high-quality juveniles. Even though several commercial diets are currently available in the market, the room for optimizing a weaning diet for shrimp larvae/post-larvae is still large. This work aims to summarize the roadmap followed to develop a premium and cost-effective microdiet boosting shrimp post-larval performance. Firstly the protein requirement at the post-larval stage was evaluated. Protein sources were subsequently tested and the cost-effectiveness of 3 microdiet prototypes was finally assessed. Growth, survival and economical conversion ratio were used as criteria to select a microdiet for shrimp post-larvae with potential for market deployment.

Materials and methods

Three trials where conducted under the scope of project SHELLWIN, where white leg shrimp post-larvae (initial wet weight 3-13 mg) were reared under standard procedures in triplicate tanks (0.28 m2). Shrimp reared at RIASEARCH Lda facilities (Portugal), at 27-28ºC, and fed on microdiets ad libitum for 21 days. For the protein requirement study, 6 diets ranging from 34 to 63% crude protein were used, composed by fish meal, fish protein hydrolysate, wheat gluten, wheat meal, squid meal, fish oil, vitamin and mineral premixes and cholesterol as main ingredients. In the protein sources trial, diets containing fish meal, squid meal, krill meal or a balanced mix of these three (52% crude protein), as main ingredients where tested. For the cost-effectiveness assessment, the selected microdiets were: a standard diet (A); a premium diet with a cost around 53% higher than diet A; and an ultra-premium diet with a cost 144% higher than diet A.

Results

Protein requirement trial: a dose-response pattern was established for shrimp postlarvae (3-70 mg wet weight), with a break-point 47.1% dietary crude protein (Figure 1). No significant differences were observed for survival (above 80%) or FCR (below 1.5) in most (44-63% crude protein) tested levels, except for the lowest (34% crude protein).

Protein sources trial: No significant differences between treatments were observed regarding final body weight (Figure 2). Survival was significantly lower in the diet having fish meal as the main protein source.

Cost-effectiveness trial: No significant differences were obtained for post-larval survival, with high results being achieved for all dietary treatments (75 - 87%). Shrimp post-larvae fed the ultra-premium diet achieved a significantly higher final body weight (Figure 3) and relative growth rate (19.8 %/day) than those fed the remaining diets (A – 15.6 %/day; B – 17.1 %/day). No significant differences were found regarding ECR, even though diet B tended to have a higher value.

Conclusions

The results obtained in the SHELLWIN project show that:

1) A dietary protein requirement of 47.1% was established for shrimp postlarvae (3-70 mg wet weight).
2) Squid and krill meals proved to be good sources of protein for the early development of shrimp, as well a mixture of these two with a high quality fish meal.
3) Higher quality diets can be advantageous in early phases of production, leading to a reduction of time to reach commercial size, leading to a higher number of production cycles per year and savings in operational costs.

Acknowledgements

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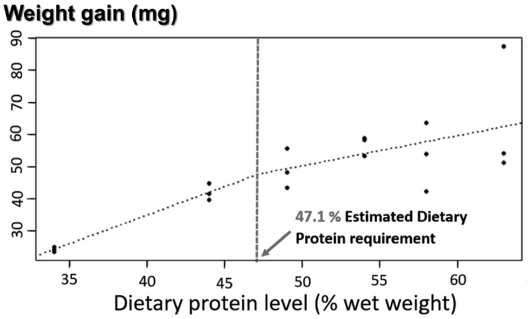

Fig. 1. Weight gain of Whiteleg shrimp (Penaeus vannamei) fed different protein levels.

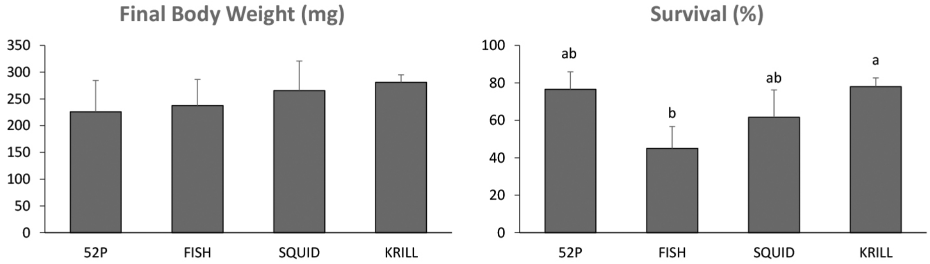

Fig. 2. Final body weight and survival of Whiteleg shrimp fed diets with different main protein sources. (P<0.05, 1 way ANOVA).

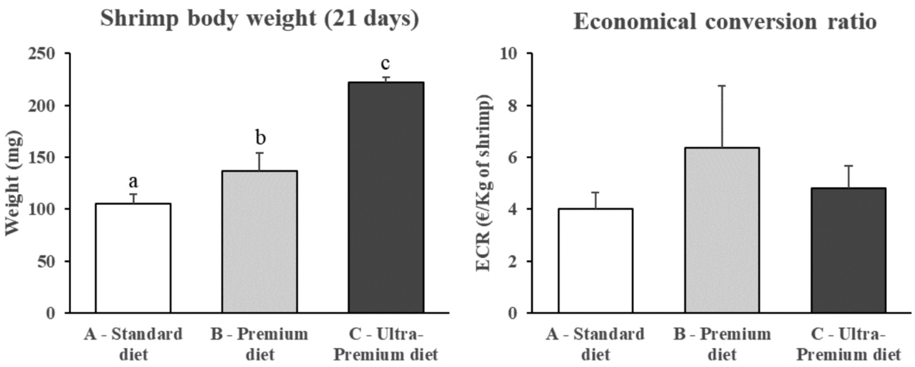

Figure 3. Shrimp final body weight at the end of the experiment and economical conversion ratio (€ spent in feed/Kg biomass gain) of the three different microdiets. (P<0.05, 1way ANOVA).

CONSERVATION OF THE BELUGA STURGEON (*Huso huso*) IN THE ADRIATIC AREA; DEFINING GUIDELINES BASED ON CONTEMPORARY AND ANCIENT GENETIC DIVERSITY

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The Beluga sturgeon (*Huso huso*) is one of the three sturgeon species historically present in the Italian waters together with the Adriatic sturgeon (*Acipenser naccarii*) and the common sturgeon (*A. sturio*). Since the 80ies the local population is extinct from the Adriatic area, but the species is still present in the Black, Azov and Caspian Seas and in their main tributaries. In Italy, as well as in many other countries, it is well represented in many aquaculture plants where it is reared for caviar production.

After the many restocking efforts conducted in Italy for the Adriatic Sea, the Beluga sturgeon is becoming the object of different conservation projects. The growing interest for the reintroduction of the Beluga sturgeon in the Po River basin is involving several actors who, with different methods and different strategies, act independently.

With the aim of providing informed criteria for the reintroduction of Beluga in Italian rivers we analysed its contemporary and ancient diversity based on a multi-markers approach. Genetic analyses on the mitochondrial d-Loop, 27 nuclear microsatellites and 893 SNPs isolated through the 2bRAD were conducted on 119 individuals from 3 geographical basins (Azov, Black and Caspian seas). Mitochondrial information was also used to evaluate the variability of the extinct Adriatic population by analysing a few available museum samples. The historical Adriatic sample cannot be traced back to any of the contemporary ones raising the hypothesis of the presence of a past isolated population.

More in general the genomic approach allowed us to depict for the first time a clear and supported genetic differentiation between two areas (the Black–Azov and the Caspian basins) and to identify a panel of diagnostic SNP loci suitable for geographic allocation. We provided guidelines for the upcoming proper restoration of the Italian extinct Beluga population and for any future management activity of this species in its entire distribution range.

In synthesis, this contribution has the purpose of a) briefly introducing the state of the species in its distribution area, b) reporting the results of a recently conducted analyses of the geographical pattern of genetic variability across the whole distribution area, with the aim of providing and discuss useful information for an informed planning of conservation activities; d) highlighting the need of a coordinated national and international strategy for the restoring of the Beluga sturgeon.-

HAS THE LPA SYSTEM BEEN SUCCESSFUL? SMALL-SCALE AQUACULTURE RECRUITMENT IN MAINE, USA, THROUGH THE LIMITED-PURPOSE AQUACULTURE (“LPA”) LICENSING SYSTEM

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The system for leasing publicly owned submerged marine lands in the state of Maine in the United States for aquaculture development concerns the recruitment of ocean farmers there into marine aquaculture. Maine’s “Limited-Purpose Aquaculture” (LPA) license was designed to induce small-scale farmers to enter the industry to experiment with a variety of means for achieving economic sustainability. This study analyzed recruitment of new ocean farmers into small-scale, low-trophic level (LTL) marine aquaculture in Maine through the LPA system. Through an online survey (n = 72) and a focus group (n = 7) of LPA-holders, data was collected on holders’ experiences in sustaining their operations, which encompass costs-benefits analyses as well as issues of access to working waterfronts, equipment, expertise, and various markets. Sixty-seven percent (67%) of respondents indicated that they sold their LPA products, and 32% of those not selling their products reported that they wanted to sell them. Fifty-eight (58%) of respondents wanted to expand their aquaculture operations to scale beyond the LPA license parameters; 61% expressed discontent with the size of their farm. Survey and focus-group data demonstrated that although 85% of LPA holders felt that their experiences in the LPA system had allowed them to make informed decisions about whether to expand their farms, many were concerned about the administrative and resource barriers they faced in scaling up their operations.

DISEASES RISK ASSESSMENT ASSOCIATED TO THE MOST RELEVANT INFECTIOUS AND PARASITIC DISEASES IN SEABREAM AND SEABASS FARMING SYSTEMS IN EUROPE

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Introduction:
Prevention is one of the most relevant aspects to provide efficient tools for improving health management in aquaculture. However, establishing useful guidelines for this purpose is not an easy task since the development of diseases in fish are usually multifactorial. The combination and impact of different factors, such as the characteristics of the infectious agents and fish host, environmental conditions, geographical parameters, and farming systems amongst others represents the main elements that determine the risk or likelihood to be impacted by a certain disease. The knowledge of these factors allows to establish biosecurity and prophylactic measures to reduce the risks of emergence of pathogens and the susceptibility to infectious diseases.

Thus, the aim of this study is to assess the relevance of these factors in order to identify the main risk factors for the most relevant diseases in gilthead seabream and European seabass in Europe

Methods:
The relevance of the different diseases of farmed gilthead seabream and European seabass in the European Mediterranean farms was evaluated after an accurate review of the available literature and discussed amongst a panel of fish health specialists from the e PerformFISH consortium. Once diseases were selected, the risks concerning each disease were also identified by a specialist working team formed by scientists using a HAZOP (Hazard and Operability Study) evaluation system, combined with the information available from technical and scientific literature for each disease, as well as from the personal clinical experience of the members of the working team. After this preliminary internal assessment, each risk factor associated to specific diseases and relevant production phases was scored by a broader panel of experts using DELPHI techniques. Experts had to assess each risk recognized by the team of specialists from 1 to 5, depending on its relevance to face problems with the disease or to avoid them.

Results:
The main diseases of gilthead seabream and European seabass selected by specialist in the Mediterranean Sea were Viral Nervous Necrosis (VNN), Photobacteriosis and disease caused by *Sparicotyle*. The main risks selected by specialists associated to these diseases were grouped in three categories: those related to farm environment (water supply, environmental conditions, epidemiological status of the farm, location…), general management (husbandry procedures, feeding, origin and control of new fish…) and risks associated with the management to avoid a specific disease (efficient protocols to detect pathogens, treatments, vaccination…).

Based on these results, dedicated well-trained personnel and with high awareness of well-established biosafety protocols was considered the most relevant factor to avoid the appearance of VNN in a facility. This recommendation was common to all the production phases. Concerning risks, the supplementation of broodstock with wild fish was the riskiest practice for VNN in broodstock facilities, and the origin of the eggs and juveniles from an external supplier with no specific VNN-health certification were identified as the main risk factors in the other phases of the production system. However, in the case of ongrowing, the highest score on VNN sanitary risks was related to the location of the cages, considering the farms located in a VNN endemic area and with water temperatures above 25 °C the ones with highest VNN risk. On the other hand, there was an agreement that one of the most beneficial practices to avoid VNN infections in both cages and tanks production systems in ongrowing was the systematic vaccination with VNN commercial vaccines following the manufacturer's recommendations.

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Vaccination was also considered the most beneficial practice to avoid Photobacteriosis in ongrowing cages, especially if fry vaccinated with bath and IP booster, in all fish stocks, and using of autologous vaccines instead of commercial vaccines. A rapid action by feeding with medicated feeds immediately after the detection and identification of the outbreak is also considered a good practice to avoid infections in this case. The main risks considered by the experts related to Photobacteriosis are associated to the farm environment, such as the location of the farm in a Photobacteriosis endemic area and/or with frequent or sudden thermal oscillations.

Finally, similar to bacterial disease, the farm environment has an essential role in the appearance of problems with *Sparicotyle*. In this case, to place the farm in a shallow area with weak water currents and/or with a large number of seabream (escapees) around the cages are the main risk factors, but also the management of the farm, if no year-class distribution is applied. A specific management for *Sparicotyle* is strongly recommended to avoid problems with this parasite, for example periodically net changing in several cages and according to *Sparicotyle* infection levels in the farm.

Conclusion:

Taking into account all the considerations and scores by specialist and experts, seems that biosecurity is the best strategy to avoid infectious problems in broodstocks, hatcheries and nurseries, meanwhile, vaccination and management are the keys to reduce the potential impact of the disease in ongrowing stages.

BEHAVIORAL RESPONSES TO DIFFERENT FEED ADDITIVES IN ZEBRAFISH (*Danio rerio*) LARVAE: A PRELIMINARY STUDY

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Introduction

Feed losses are a serious problem for the aquaculture industry, as feed represents 40–75% of production costs in this sector. In addition, the release of large amounts of organic matter into the environment leads to a significant increase of nitrogen (N) and phosphorus (P) concentration promoting eutrophication problems. As a consequence, a reduction in waste outputs is now mandatory and this goal can be achieved by a better management of feeding activities and by improving aquafeed palatability through specific feed additives.

The present study wants to explore the application of feed additives for aquafeed formulations, possibly increasing palatability and reducing, at the same time, the above-mentioned issues. A set of different products have been tested during a behavioural test performed on zebrafish (*Danio rerio*) larvae to identify those that positively attracted the fish.

Materials and Methods

Eight hundred zebrafish larvae aged 25 days post fertilization (dpf) were divided in forty groups of 20 individuals each and fish were singularly exposed (after a 30 mins acclimatization period) to a different water-soluble olfactory cue (named from P01- P40). Tests were performed in rectangular white plastic tanks (L 24 cm, H 8 cm, W 8 cm; water level: 3cm), uniformly lit by a LED stripe using a Canon LEGRIA HF R38 camera to record the behaviour of the fish during testing time. During each test, two cellulose sponges (side 1 cm), mounted on a detachable metal stand, were inserted into the tanks: one contained the olfactory cue (1 %) while the second water and served as control for object exploration. During the experiment fish were fed at different times, and behavioural tests (15 mins each) were performed one hour after each feeding to exclude foraging behaviours caused by hunger and to obtain a homogeneous result excluding behaviours due to the circadian rhythm. All video recordings were visualized and analysed using BORIS software. A two-step analysis was performed: one-way ANOVA was firstly used considering the total time of analysis (min 0-15) to evaluate whether the larvae responded differently to several olfactory cues; then, the data of the first 5 minutes (min 0-5) and the total time recorded (min 0-15) were analysed separately by computing the overall proportion of time spent close to the novel olfactory cue in these two periods of time. One-sample t-tests against the preference expected by chance (0.5) were used to test whether the preference for the novel olfactory cue was different from random choice.

Results and discussion

Considering the total time of analysis (min 0-15), the percentage of time spent by the fish in the sector with the novel olfactory cue significantly varied according to the type of odour used (ANOVA, $F_{39,724} = 2.42$, $p = <0.0001$, $= 0.12$). The average percentage of time spent in the sector with the odour are showed in Figure; the olfactory cues that showed a significant effect (one-sample t-test) are highlighted in the Figure. The two temporal analyses revealed that in some cases, same olfactory cues showed different effects in zebrafish larvae behaviour. Analysing different time slots is essential because the effects of each olfactory cue can vary during the time in relation to its chemical nature and dilution factor. As for example, novel or too much concentrated odours can initially act as alarm signals evoking avoidance or defensive behaviours. However, no one of the tested odours showed this behaviour. As regards the time effect on fish behaviour, the present study evidenced that: i) P02 and P35 odours showed a constant attractive effect; ii) P25 odour instead, showed attractive effect only during the first five minutes of test; iii) the remaining odours displayed a negative or neutral effect on fish. To reduce feed losses in aquaculture, feed has to be ingested by fish quickly and thus the search of new feed additives should be focused on the olfactory cues that have a quick and positive effect on fish during the first minutes of feed administration.

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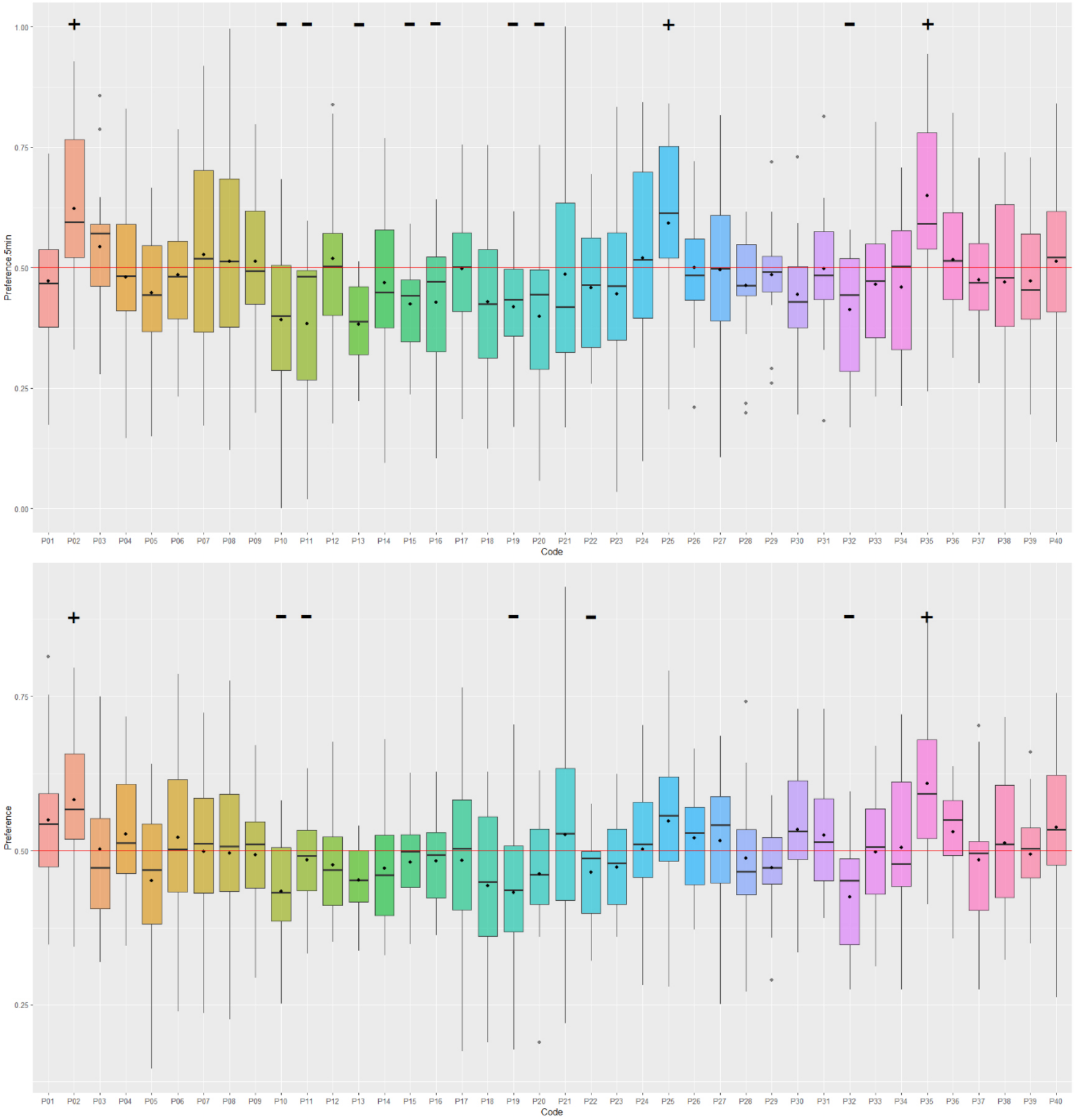

Figure - Boxplot of minutes 0-5 and 0-15 respectively, showing the average percentage of time spent by fish in the sector with the specific olfactory cue. Odours that showed a significant (positive +; negative -) effect are highlighted in the figure.

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EFFECTS OF FEEDING TIME ON THE DAILY RHYTHMS OF TEMPERATURE SELECTION IN THE BLIND MEXICAN CAVEFISH (*Astyanax mexicanus)*

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Animals and plants live on a rhythmic planet, with regular and predictable periods of light-darkness, temperature and food. As a result, the evolution has selected the existence of endogenous circadian clocks that synchronise their physiology and behaviour with the environmental cycles.

In addition, besides the existence of daily cycles of temperature in the wild, called thermocycle, fish display a thermal preference and the ability to discriminate between different temperatures. Temperature selection in fish is connected to their biological processes and they are free to choose the best temperature in which the development of these processes will be most effective.

Over the past few millions of years, the blind cavefish *Astyanax mexicanus* has been isolated from neighbouring rivers in underground caves in Mexico. These fish have evolved and adapted to a life in complete darkness and these adaptations include the loss of eyes and pigment, as well as changes in metabolic rates, activity and the loss of sleep activity/circadian rhythms to varying degrees (Steindal et al., 2018).

The aim of this study was to investigate the existence of daily rhythms of temperature selection and the effect of feeding time on these rhythms in a fish that has evolved in an arrhythmic environment, such as *A. mexicanus*.

To achieve this objective, we used a multi-chamber aquarium and created a thermal gradient (from 20 ºC to 28 ºC) using water heaters and coolers. The fish were subjected to a 12 hours of light and 12 hours of dark photoperiod (LD 12:12 h) and fed according to 4 different feeding schedules: random (RD), feeding in the middle of the light phase (mid-light, ML = ZT 6 h), feeding in the middle of the dark phase (mid-dark, MD = ZT 18 h) and fasting. Daily temperature selection was determined by video analysis of animal behaviour. A constant temperature of 24 ºC in the whole system was used as control.

The fish showed a daily rhythm of thermal preference, regardless of the feeding protocol. Specifically, they selected warmer temperatures during the light phase and cooler temperatures during the dark phase.

In addition, during acclimation (first 3 days that preceded the experimental phase), the fish selected a higher mean temperature than that observed in the other experimental phases, showing a possible emotional fever in response to the new environment (Rey et al., 2015). Fish fed at ML tend to go to lower temperatures in the hours before feeding and warmer temperatures afterwards. During the fasting phase, the selection rhythm has a lower amplitude but still significant ($p < 0,05$).

These results show how important thermal preference rhythms can be, even in relation to feeding time, also in a species adapted to an almost totally arrhythmic environment.

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Acknowledgments

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NOVEL PROTEINS IN SALMON FEED: A SURVEY OF SWEDISH CONSUMER ATTITUDES

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Introduction: Study background

Traditional sources of protein in fish feed for salmonids, such as fishmeal, face sustainability challenges in terms of environmental and marine wildlife conservation [1]. As such, the feed industry is increasingly investing in using alternative ingredients with high protein content in future fishmeal and animal feeds. Alternative sources of protein for fish (salmonids) feed is an emerging research and commercial field. While findings in food research indicate that consumers perceive fish as healthy and natural, when new products and technologies are to be launched on the market, the degree of its success on the market depends on consumer perception, knowledge, and acceptance of the product, which influence purchase preferences [2]. As feed industry is intensively working on new possible protein sources, such as insects, microalgae, bacterial proteins, and proteins from food by-products, there is therefore a need to map consumer attitudes to these new sources to gauge consumer acceptance levels. To this end, this study investigates consumer acceptance of novel proteins in fish feed innovations. Our findings uncover Nordic consumer attitudes and preferences for types of novel protein feed for salmonids.

Data collection

Respondents were approached in three main ways, (i) via social media platforms such as Facebook, LinkedIn and Instagram messaging systems, (ii) via personal social network referrals and (iii) via face-to-face impromptu meeting requests in public spaces of various social context settings such as restaurants, grocery shops, boutiques and outdoor parks. A total of 30 respondents (17-70 years old) agreed to be part of this study. The majority (36) respondents were Swedes who resided along the Swedish West coast.

Method

The respondents were interviewed using a set of open, semi-structured questions constructed as a means of an interview guideline. The objective of the interviews was eliciting as much feedback as possible from the respondents. The respondents were encouraged to speak openly about salmon feed in farmed salmon in general, and attitudes towards alternative protein sources. The questions were designed from a systems thinking approach [3,4] to collect information along four broad dimensions, with different knowledge dimensions (i) Individual, (ii) Product, (iii) Social and (iv) Environmental. Each dimension reflects a broader context of knowledge than the previous dimension, with questions pertaining to the environmental dimension encompassing the broadest respondent perspective. The design of the interview questions aims at collecting a holistic overview of how respondents view farmed salmon and using alternative proteins in salmon feed. The interviews were on average 45 minutes long. All interviews were transcribed in accordance with the Gothenburg Transcription Standard 6.4 [5].

Findings and discussion

The subject of salmon feed and alternative proteins in salmon feed came across as a niche and unfamiliar subject for many of the respondents. Only 4 respondents out of 30, who were in the fields of higher education, research, related aquaculture activities, or hobby fly fishing, could be said to have some knowledge on farmed salmon and salmon feed. Most had no insight into salmon feed issues and remained unaware that most salmon sold on the market in Sweden is from Norway. Animal welfare was a greater concern, and some respondents said that if the salmon fared well on alternative protein feeds, that they would be fine with buying and eating the salmon. The respondents also expressed interest to have more information about salmon feed in general, and novel proteins in salmon feed. The respondents also reported to be willing to pay more for the salmon that is sustainably farmed and fed a salmon appropriate diet. The respondents were most positive towards insects as a novel protein in salmon feed. In general, all respondents are increasingly sustainability conscious, and reported clear preferences for buying the salmon or seafood that is sustainable, fed with a high quality protein, with a lower carbon footprint.

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Practical business implications

The findings indicate limited consumer knowledge about salmon feed and origin of salmon. As this information is currently not provided on product labels, providing informative product labels to consumers could be considered by the industry. Further, as sustainability is important to consumers, informing the consumers about sustainable feed could benefit product sustainability branding.

Respondent feedbacks also indicate the following potential areas of value-added exploitation for the salmon industry such as: (i) exclusive branding and marketing salmon based on higher quality, more sustainable feed based on insects instead of soya beans or other vegetable proteins; product differentiation for Norwegian salmon based on high quality protein salmon feed; (ii) introduction of appropriate labels, and making information for consumers available on Norwegian produced salmon, raised on high quality protein feeds; (iii) exploiting high quality insect-based salmon feed as a product differentiation strategy for salmon produced in Norway, together with high sustainability standards for Norwegian salmon farming; and (iv) improved research and product development opportunities for flavour and texture profile when salmon are fed higher quality protein feeds.

Future research

Two topics that arose from the respondents that were of interest for consumers were (i) need for increased information on the origin of the salmon, and what it was fed on package labels and (ii) using entire supply chain sustainability as an accounting measure of how sustainable the salmon is as a food product.

Acknowledgement

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SENSITIVITY AND VARIABILITY IN BIOPHYSICAL MODELS OF MUSSEL LARVAE IN SCOTLAND

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Introduction

Molluscs, mainly bivalves, represent a significant portion of our current aquaculture production worldwide accounting for over 22% of the total global harvest (FAO, 2018).The ability of mussels to disperse and settle on suitable substrates is exploited by the aquaculture industry for the seasonal supply of seeds. However, dispersal of *Mytilus spp.* larvae at present remains largely unresolved and it is difficult to measure in situ. In this study, we use an unstructured 3D hydrodynamic model to drive a particle tracking model where prediction of larvae movement and dispersal, together with simulations of spat dispersal in designated localities help to investigate potential connectivity between sites (Adams et al., 2016). These advanced approaches help to characterise broad-scale dispersal patterns and have wide implications that can be applied to evaluate the efficiency of spatial-temporal management measures.

Material and Methods

The biophysical model is based on an unstructured grid approach; modelling pelagic connectivity between aquaculture sites consists of a hydrodynamic model, a particle tracking model and post-processing module to estimate the source location of the mussel larvae. The domain for this study covers the west coast of Scotland (Aleynik et al., 2016), building upon previous work using smaller domains in the same locality (Adams et al., 2012, Adams et al., 2014, Aleynik et al., 2016, Davidson et al., 2021). The hydrodynamic model applied for this study is based on the Finite Volume Coastal Ocean Model (FVCOM; Chen, 2013). Particle tracking was carried out using the model of Adams et al. (2012, 2016). This model, originally developed to predict the dispersal of sea lice, links physical processes such as water movements with biological processes such as maturation and mortality. The movement of larvae incorporates advection due to local currents and a fixed random diffusion term.

We run the model under two different scenarios. First a single day release of particles in order to assess variability in predicted dispersal patterns. And second a tidal cycle release of particles to quantify the accumulation of particles in each target location (Shellfish farms) and to detect possible source locations seeding each target location.

Results

We have analysed the general patterns related to the connectivity between source areas and different geographical areas. The overall overview from the single day releases shows a constant pattern between years (2017 to 2021) and different depths (2 m, 6 m, and 10 m depth). Through the tidal cycle releases, we have calculated the particle dispersal accumulation and we've been able to identify possible source locations of mussel larvae seeding our target locations (Shellfish farms located on the West Coast of Scotland) (fig.1). Results were considered in context with the relevant wind roses for the simulated periods. We have produced monthly representation of the current direction for the same period. With this study we are one step closer to help mussel farmers with their site selection process, as well as to understand the larvae movements through the flow along the complex coastline and bathymetry in fjordic coastal environments.

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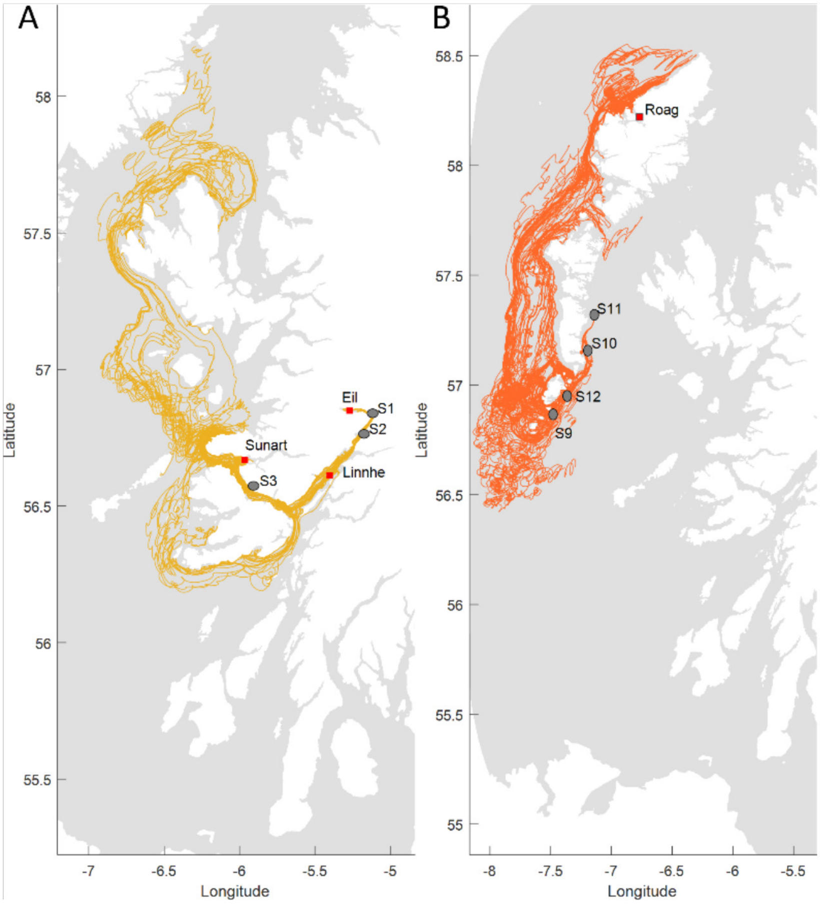

Figure 1. West Coast of Scotland; red squares are the target locations and grey circles the source locations. Figure 6A shows an example of self-recruiting where S1 is the only source location for Loch Eil. And self-recruiting and influence of external recruitment where S1 and S2 are source locations for Loch Linnhe and S1, S2, and S3 for Loch Sunart. Figure 6B shows an example of not self-recruitment for Loch Roag.

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HISTOCHEMICAL AND HISTOLOGICAL CHANGES DURING OVARIAN DEVELOPMENT OF RIVER PRAWN, *Macrobrachium americanum* SPENCE BATE, 1868

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In this study, the reproductive biology in the natural environment through histological and histochemical techniques that describes the gonadal development or oogenesis during the reproductive season of female river prawn *Macrobrachium americanum* in B.C.S. Mex. Specimens were collected the month July-October. Four stages of gonadal development were characterized: previtellogenesis I and *II*, vitellogenesis *I* and *II* and eight sub-stages: oogonia (Og), nucleolus chromatin (Cn),oocyte early perinucleolus (Opn1), oocyte late perinucleolus (Opn2), oocyte lipidic droplet (Old) (Li2), oocyte early vitellogenic (Ov1), oocyte late vitellogenic (Ov2) and germinal vesicle migrand (Vgm) (Fig. 1). The criterion used was the measuring oocyte size (µm), the nucleus and the characteristics of the ooplasm and the accumulation of yolk (lipids, carbohydrates, and proteins were quantified in the different stages) showed significant differences ($P<0.05$). The number of biomolecules that constitute yolk, such as triglycerides, carbohydrates and proteins showed significant differences between the different oocyte types, presenting the lowest values of triglycerides (5.42 ±1.21%) in the previtellogenesis and the highest values in vitellogenesis. This proportion is inverted in phospholipids (54.92±1.40%). Establish that the female is a partial spawners. This information might help for conservation measures in the species, as well as to establish the fishing period of the species.

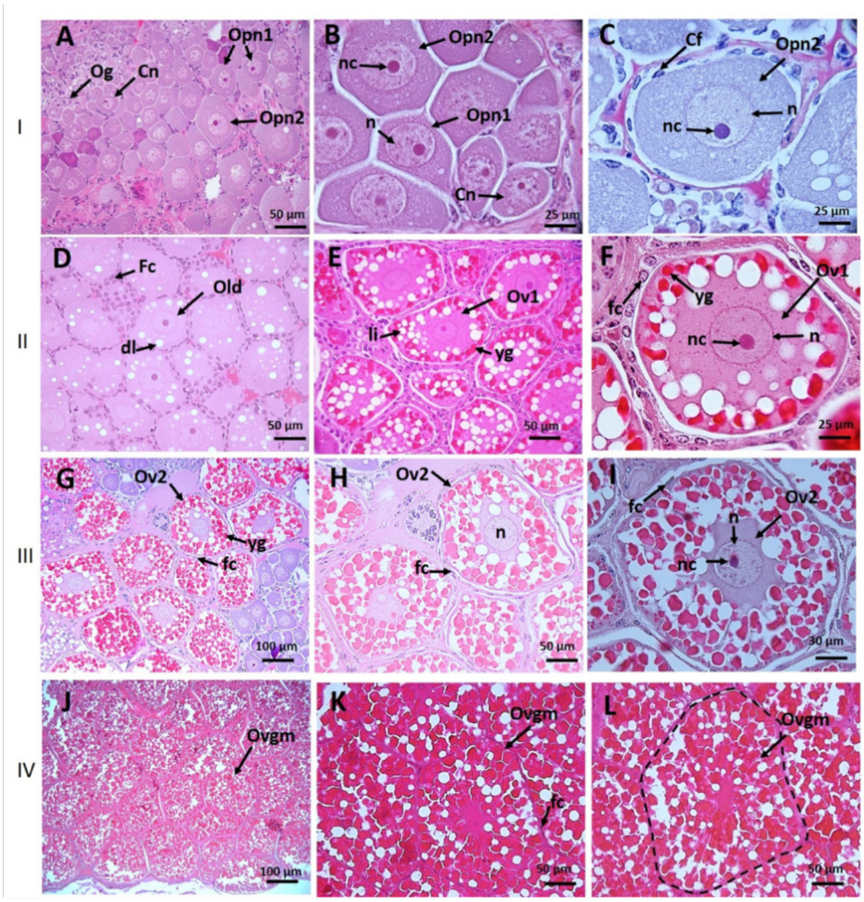

Figure 1 Stages of ovarian development of *Macrobrachium americanum*.

PHYTOBLOOM® RAS FORMULA: DEVELOPMENT OF A NEW DIET DESIGNED FOR ROTIFERS RECIRCULATION AQUACULTURE SYSTEMS

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Introduction

Recirculation aquaculture systems (RAS) are more sustainable, when compared to conventional production modes, due to the reduction of water usage simultaneously maintaining the water quality. This aquaculture system started recently to be applied in intensive rotifers production. Rotifers (*Brachionus plicatilis*) production is widely constrained by degradation of the water quality and the fluctuation of environmental parameters (e.g. oxygen, temperature, bacterial load, ammonia) in the tank. Therefore, the use of RAS systems (e.g. RotiRas, IDEE aquaculture, France), allowed to improve the rotifers population densities and outputs. *Nannochlorpsis* sp. is nutritionally balanced and successfully used for rotifers nutrition. Rotifers high-density cultures require a hight daily input of microalgae and commercial microalgae products are the solution to feed rotifers in RAS systems under semi-continuous mode of production. However, the constricted filtering system do not allow the application of product that generate high microalgae agglomeration. The use of low density *Nannochlorpsis* sp. products prepared with low salinity solutions may reduced the agglomeration and avoid filter clogging. The objective of this work was to develop a new microalgae product specifically designed for rotifer nutrition in RAS systems under semi-continuous mode operation.

Material and methods

The product RAS, liquid concentrate is formulated with industrially produced *Nannochloropsis oceanica* paste prepared with a saline solution of 35ppt, to obtain a final product with 15% of dry weight. The formation of microalgae agglomerates was evaluated and the dimension of the agglomerates. The microbiological quality (PCA, TSA and TCBS media) of the product was monitored throughout storage time (1-3 months at -20ºC) and post-thaw days (3-7 days at 4ºC). The product was applied in a RotiRas (IDEE aquaculture, France) production system with a semi-continuous production mode, the target daily harvest was 500 million rotifers. The rotifers were cultured with a density of 1500 - 2000 rots/mL, at 28ºC, 20ppt of salinity, maintaining the culture pH between 7.5 to 8 and ammonia below 1mg/L. There was a daily exchange of 50% of the water of the culture. The efficiency of rotifers production of RAS formula was compared with an available commercial product (CP1). The dosage of RAS formula was 2ml of product per million of rotifers. For CP1 product the recommended daily dosage was respected. Rotifers concentration and percentage of rotifers with eggs were evaluated daily.

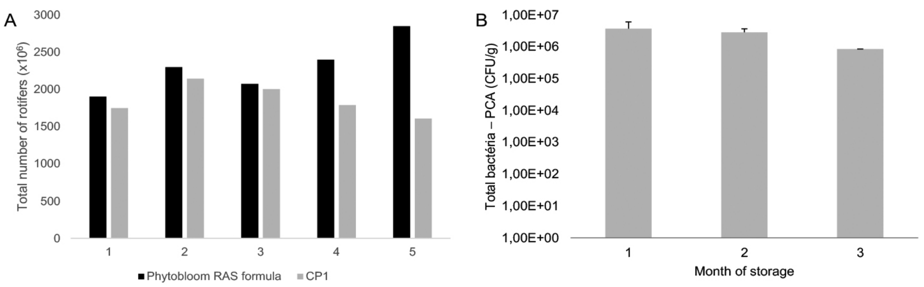

Fig 1 – Rotifers new diet Phytobloom® RAS Formula and commercial diet 1 (CP1) quality characterization in relation to A) rotifers total daily production in RAS systems with a 700 million rotifers of daily harvest and B) Phytobloom® RAS Formula total bacteria throughout storage (-20ºC) time.

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Results and Discussion

RAS formula was successfully applied in the RAS system. The rotifers concentration was maintained at 2075 rot/ml with a daily harvest of 700 million rotifers. Therefore, the total number of rotifers was maintained according to the previously established target (Figure 1A). The total bacteria of RAS formula was maintained throughout storage time (Figure 1B), therefore the low salinity of the product did not affect the product microbiological quality in the first 3 months of storage.

The RAS formula reached an average of 30±12% of rotifers with eggs and CP1 product reached an average of 33±4% of rotifers with eggs. In conclusion, the new Phytobloom® RAS Formula was successfully applied in rotifers RAS system, without filter clogging and reaching the target rotifers densities and daily harvest.

Acknowledgements

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ACCELERATING THE DEVELOPMENT OF MARINE AQUACULTURE IN THE NEARSHORE: SCALING OUT IS AS IMPORTANT AS SCALING UP

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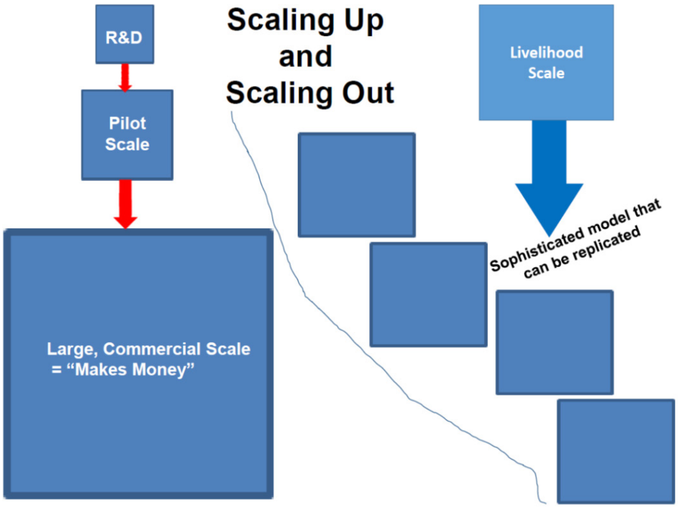
Nearshore oceans with their high primary and secondary productivities, fluid logistics and close proximities to markets are excellent places to develop marine aquaculture. However, as common property resources, the numbers/diversities of user conflicts increase with proximity to shore. Conflicts result in the overuse of the precautionary approach by inadequate ocean governance which limit increases in the number/sizes of marine aquaculture operations nearshore. Sustainable system change to gain easier access to expand marine aquaculture nearshore would consider ocean engineering advances made offshore that can open large areas of energetic, nearshore oceans with less user conflicts. Governance systems would then include options for accelerated permitting in these areas as well as differentiate between scaling "out" and scaling "up". To illustrate changes needed the case study of the state of Maine USA and its contiguous sea, the Gulf of Maine (GoM), are discussed. The GoM is a productive marine basin with potential for the development of both fed and non-fed aquaculture in a bioregion that has an historical affinity for seafoods. Markets are abundant as consumers have a higher rate of seafood consumption than national averages but the region meets its seafood needs from imports. If a straight line were drawn along the outer edges of Maine's islands/bays, a distance of ~360 km to Canada and extending ~4 km from shore, ~1700 km^2 of highly energetic nearshore space is available with less user conflicts. However, scaling up in these new ocean spaces remains regulated though a leasing structure tiered by size whose upper bounds are derived more by social/political factors and a "social license to operate". Maine has the unique system to "scale out" marine aquaculture development, the limited permit access (LPA) system, which as of end 2021 attracted over 700 entrants. A new governance vision for scaling out in nearshore energetic oceans is required that can merge ocean leasing systems for businesses to scale up and scale out that can limit overuse of the precautionary approach and reform nearshore marine aquaculture policies.



DIETARY EXOGENOUS ENZYMES SUPPLEMENTATION IN PLANT-BASED FISH DIETS AFFECTS POSITIVELY THE INTESTINAL ENDOGENOUS ANTIOXIDANT AND DIGESTIVE ENZYME ACTIVITIES OF THE EUROPEAN SEABASS JUVENILES

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Introduction

The European seabass (*Dicentrachus labrax*), a carnivorous marine species, is one of the most important species for Mediterranean aquaculture. As it has already been demonstrated, *D. labrax* can be fed on terrestrial plant ingredient-based fish diets (PM) without compromising its growth and feed utilization (Kaushik *et al*., 2004). However, most of these plants contain several anti-nutritional factors (ANTs) that are not heat-labile and/or inactivated by feed processing and therefore can affect the nutrient digestibility, growth performance and gut health of this species (*Torrecillas et al*., 2017). Nonetheless, an effective way of eliminating the negative effects of ANTs on this fish and improving digestibility of plant ingredient-based diets is the supplementation of exogenous enzymes in the feedstuff (Magalhaes *et al*., 2018). In omnivorous species exogenous enzyme supplementation can promote the secretion of the endogenous enzymes in fish and thus, boosting the digestibility of nutrients via raising activities of intestinal digestive enzymes (Drew *et al*., 2005; Farhangi and Carter 2007; Lin *et al*., 2007). However, data concerning the effects of supplemented exogenous enzymes on *D. labrax* juveniles' intestinal digestive enzymes is limited, while there is no information of any effects on their intestinal antioxidant enzyme system.

Materials and Methods

Four isoproteic (48%), isolipidic (16%) and isoenergetic (21%) diets were formulated and produced at our lab (IMBBC-HCMR). Briefly, a 20% fish meal-based formulated diet was stated as FM diet while a diet of 30% plant ingredients-based consisting of rapeseed, sunflower and guar meals at inclusions levels of 10% each as PM diet. Then, to the PM diet three exogenous enzyme products were supplemented to generate two more diets: a diet with a 0.05% of a solid-state fermentation supplement (SSF) which contained a heterogeneous complex of residual enzymes liberated by the action of a fungus on the substrate during fermentation and a diet with a mixture of 0.01% phytase plus 0.03 % xylanase (Phyt/Xyl mix). Following a three-month experimental feeding trial, the specific activities of the antioxidant (CAT, Se-GPx, GST) and digestive enzymes (Trypsin, Aminopeptidase, ALP) were measured in the whole intestines of the fish. Correlation was also performed with measured critical growth performance indicators (i.e. weight gain, feed intake, SGR and FCR).

Results

Table 1. Growth performance indicators of *D. labrax* fed on plant ingredient-based diets supplemented with exogenous enzymes.

	FM	PM	SSF	Phyt/Xyl mix
Weight gain (g)	40.3 ± 2.4 **a**	20.9 ± 0.4 **b**	27.2 ± 0.8 **c**	33.2 ± 0.3 **d**
Feed intake (%)	1.73 ± 0.04 **ab**	1.61 ± 0.05 **c**	1.71 ± 0.01 **ab**	1.79 ± 0.07 **b**
SGR	1.35 ± 0.05 **a**	0.88 ± 0.01 **b**	1.05 ± 0.03 **c**	1.20 ± 0.00 **d**
FCR	1.21 ± 0.02 **a**	1.64 ± 0.05 **c**	1.48 ± 0.03 **bc**	1.38 ± 0.05 **b**

**the different small letters indicate significant differences at p < 0,05 level*

Table 2. Intestinal antioxidant and digestive enzyme activities of *D. labrax* juveniles fed on plant ingredient-based diets supplemented with exogenous enzymes.

Specific Enzymes Activities *(U/min/mg prot*	FM	PM	SSF	Phyt/Xyl mix
Antioxidants				
CAT	335.6 ± 40.4 **a**	32.0 ± .,9 **b**	125.5 ± 9.8 **c**	181.7 ± 35.2 **d**
Se-GPx	252.8 ± 23.5 **a**	114.7 ± 18.7 **b**	178.1 ± 2.,4 **c**	133.8 ± 18.5 **bc**
GST	78.26 ± 8.42 **a**	35.79 ± 6.07 **b**	92.41 ± 17.56 **a**	41.19 ± 6.06 **b**
Digestive				
Trypsin	16.30 ± 2.11 **a**	10.15 ± 1.07 **c**	15.8 ± 2.18 **ab**	4.20 ± 0.67 **d**
Aminopeptidase	151.7 ± 12.,2 **a**	118.3 ± 13.3 **b**	153.9 ± 14.4 **a**	94.5 ± 11.5 **c**
ALP	92.1 ± 17.6 **a**	27.7 ± 5.5 **b**	46.8 ± 8.5 **c**	33.7 ± 7.2 **b**

**the different small letters indicate significant differences at p < 0,05 level*

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Conclusions

- Intestinal antioxidant enzyme activities were induced with the SSF and Phyt/Xyl mix diets in comparison to PM diet but were significantly higher with the SSF than with the Phyt/Xyl diet except CAT. Inductions, however, did not reach the levels of the FM diet except GST with the SSF diet.
- Digestive enzyme activities were significantly induced only with the SSF compared to PM diet and were similar to FM diet except with ALP. With Phyt/Xyl mix diet the enzyme activities were significantly lower than that with the SSF diet.
- Critical growth performance indicators were significantly improved compared to PM diet. The significant differences indicated between SSF and Phyt/Xyl mix did not coincide with the growth performance indicators, thus further research is needed.

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INTEGRATION OF SEA CUCUMBERS TO ABALONE PRODUCTION: LOW TROPHIC SPECIES IMTA SYSTEMS

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Introduction
The interest of Holothurians as deposit feeders has been demonstrated in different studies and their environmental value, through their mitigation ability, was highlighted within different IMTA systems (Hannah et al., 2013; Kang et al., 2003; Zhou, 2006) indicating that sea cucumbers can optimise the net use of wastes (Reid et al., 2013).

Given the existing research, the current studies investigated the potential for the integrated culture of the sea cucumber *Holothuria. sanctori* and *Neostichopus grammatus* with the abalone *Haliotis tuberculata* and *Haliotis midae* in order to develop an integrated Land Based IMTA production of these species.

Materials and methods

Experimental specimens
Young individuals of *H. sanctori* and *N. grammatus* were collected at a depth of 0-10 m, by scuba diving, and maintained and acclimated in experimental installations, placing them under abalone (*H. tuberculata* and *H. midae*) baskets (in which abalone were fed macroalgae and/or compound feed). After their acclimation, they were then respectively allocated to experimental IMTA units.

Adult abalone of *H. tuberculata* and *H. midae* were obtained from research and commercial facilities both at the University of Las Palmas de Gran Canaria and Wild Coast Abalone.

IMTA experimental set up
Experimental IMTA systems were designed to provide two levels compartments; one for the fed abalone and the other for sea cucumbers located below the abalone compartment. The abalone were placed in perforated baskets to allow water exchange and release of abalone wastes to reach the sea cucumbers located below.

Experimental parameters
Weight Gain (WG), and Specific Growth Rate (SGR) as well as ingestion rate, faecal production rate and water quality were analysed.

Results

The results obtained demonstrated the influence of abalone feed employed in the growth potential of the sea cucumbers and the impact on the ingestion rate. They also highlighted the potential to coculture abalone and sea cucumbers, the sea cucumber bioremediation capacity and growth under abalone as well as macroalgae production.

Discussion

The results obtained confirmed the potential to produce sea cucumbers in co-culture with abalone species as demonstrated by previous research. They highlighted the variation of sea cucumber growth potential as a function of the nature of abalone feed employed and the differences in ingestion and growth parameters according size, density and species. Moreover, the studies highlighted the potential to develop IMTA systems including only low trophic species and allowing the integration of macroalgae production in the systems. These results are of interest for the future development of low trophic species IMTA systems and the future successful integrated production of the sea cucumber species.

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Acknowledgements

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DEVELOPMENT OF STANDARDIZED *IN VIVO* WHITE SPOT SYNDROME VIRUS (WSSV) INDIVIDUAL AND GROUP CHALLENGE MODELS FOR EXPERIMENTAL INFECTION STUDIES IN *Litopenaeus vannamei* SHRIMP

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Introduction

White spot syndrome virus (WSSV) can cause a cumulative mortality up to 100% within 3-10 days on shrimp farms (Dey et al., 2020). Although progress has been made in the past 30 years, a better understanding of the pathogenesis and transmission route(s) of WSSV is still necessary. This work relies heavily on the establishment of standardized *in vivo* experimental infection models that can be used as a tool for studying WSSV pathogenicity and for evaluating the efficacy of WSSV mitigation strategies (Prior et al., 2003). A WSSV challenge model via per os route in which shrimp are individually housed has certain advantages over a WSSV group challenge model. It allows for accurate collection of research data in a highly controlled scientific setting, while increasing the throughput in selecting and developing infection control tools. The amount of WSSV positive inoculum consumed by each shrimp can be recorded meticulously. In addition, the clinical outcomes on the level of the individual shrimp can be evaluated in detail. On the other hand, a challenge in group simulates more closely the on-farm reality of a WSSV outbreak, as it allows for disease transmission between shrimp. This might indeed generate results that can potentially be easier to extrapolate to the field, but due to the less controllable nature of this experimental setting, results may be less reproducible and accurate data collection becomes more challenging. Nevertheless, both model types can be very useful tools in WSSV research. In the past, mortality levels after oral WSSV challenges have varied between studies, in part due to a lack of knowledge on the portal of WSSV entry (Prior et al., 2003; Domínguez-Borbor et al., 2019; de Gryse et al., 2020). The objective of this study was to develop standardized and reproducible individual and group challenge models for experimental WSSV infection studies in *L. vannamei* whereby 100% of clinical WSSV infection is induced. By testing different experimental conditions to obtain this clinical outcome, we aim to learn more about WSSV transmission.

Materials and Methods

Specific pathogen free (SPF) *Litopenaeus vannamei* were imported as postlarvae (PLs) from Global Blue Technology (USA) and Miami Aqua-culture (USA). Shrimp were housed in artificial seawater at 20 ppt salinity and 27°C ± 1°C. They were injected intramuscularly with the WSSV Thai-1 strain (Escobedo-Bonilla et al., 2005). WSSV positive solid inoculum was prepared from the resulting infected carcasses. This was used to infect PL65-72 shrimp through oral route. Briefly, during the 1st experiment, in which the effect of different doses within 24h was tested, shrimp were randomly divided into four challenge groups (A1, B1, C1, D1) of 15 shrimp each and one control group (Mock1) of 10 shrimp. Shrimp were housed individually in 10L tanks. The oral infection trial followed a procedure adapted from Van Thuong, et al. (2016). Over a period of 24h, individual shrimp from groups A1, B1, C1, and D1 each received respectively 4, 5, 6, or 7 doses of WSSV positive inoculum. Mock1 shrimp received 7 doses of WSSV negative inoculum. For the 2nd experiment, in which the effect of daily repeated inoculation of 4 doses was tested, shrimp were again randomly divided into four individually housed challenge groups (A2, B2, C2, D2) of 15 shrimp each and one control group (Mock2) of 10 shrimp. In A2, B2, C2 and D2, shrimp received respectively 4 doses of WSSV positive inoculum within one day for one, two, three or four consecutive days. Mock2 shrimp received 16 doses of WSSV negative inoculum within 96h. Finally, for the 3rd experiment, where the effect of group housing was tested, shrimp were randomly divided into six groups of 100 and housed in six 250L tanks at 1.5kg/m^3. Over a period of 24h, three tanks (+WSSV1, 2, 3) each received 400 doses of WSSV positive solid inoculum, while the three control tanks (-WSSV1, 2, 3) received 400 doses of WSSV negative inoculum. WSSV infection was confirmed by qPCR. The survival/mortality data were analysed statistically using the Log-rank (Mantel-Cox) test.

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Results

When shrimp from the individual challenge received either 4, 5, 6 or 7 doses of WSSV positive solid inoculum within 24h, this did not result in a significantly different mortality (A1 = 16.7±4.7%; B1 = 20.0±9.4%; C1 = 20.0±9.4%, D1 = 20.0±18.9%). However, in case of daily re-inoculation, a significantly lower mortality was observed in group A2 (28.9±16.8%) compared to group D2 (57.8±3.8%). When shrimp were housed in groups and received the same number of doses as individually housed A1 and A2 shrimp, the accumulated mortality reached 100%, while in the three control tanks all shrimp survived. For each experiment, WSSV infection was confirmed by qPCR in a sample of the dead shrimp. WSSV was absent in sampled survivors and negative controls.

Discussion and conclusion

The results of the 2nd experiment showed that the probability or risk of infection in the population increased when the inoculation procedure was repeated on subsequent days. Increasing the number of doses that are given within 24h on the other hand, did not significantly raise the chances of a clinical infection in individually housed shrimp. Moreover, the uptake of inoculum by shrimp slowed down after the 5th dose, possibly because the animals were sated. In the 1st and the 2nd experiment, a clinical infection of 100% was not reached. This level could only be reached during the group challenge. This was a striking result, because the individually housed shrimp in A1, A2, and the group-housed shrimp in +WSSV1, 2, and 3 consumed the same amount of inoculum. In addition, during the group challenge cannibalization of sick shrimp was virtually absent. How WSSV transmission exactly occurs remains unknown. Proposed routes include consumption of infected tissue, water-borne transmission, and entry via the antennal gland during urination (de Gryse et al., 2020). After analysis of the results, it was hypothesized that shrimp behaviours associated with cohabitation and high stocking densities, such as increased aggression and urination, play a major role in a WSSV outbreak.

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CO-CULTIVATION OF *Chlorella vulgaris* AND *Streptomyces rimosus*: A NOVEL PERSPECTIVE FOR ENHANCING BIOACTIVITIES OF MICROORGANISMS

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Introduction

Microalgae-bacteria symbiosis represents commensal and mutualistic inter-kingdom relationships, that may result in stimulation of algal growth, but also in the development of new metabolites with a prominent potential for application in the industry sector (Yao et al. 2018). *Chlorella vulgaris* is a green unicellular microalga with numerous biological and pharmacological properties important for human health. Streptomycetes are the most thoroughly studied microorganisms producing biologically active compounds, such as antibiotics, anticancer agents, immunosuppressives, antifungals, etc. In the recent decade, co-cultivation, as a potential replacement for mono-culturing of various microorganisms, gained special interest due to a whole range of desirable characteristics, such as modularity, robustness, predictability, scalability, and stability (Rollié et al. 2012). However, very little is known about the significance of *S. rimosus* and *C. vulgaris* co-cultures concerning the production of high-value metabolites with anti-bacterial, anti-oxidant, and anti-aging activities.

Materials and methods

C. *vulgaris* and S. *rimosus* were co-cultivated under mixotrophic conditions, with 12:12 (day:night) photoperiod on Triptic Soya Agar (TSA) plates during 7 days. Cultivated biomass was collected and freeze dried. Freeze dried biomass of C. *vulgaris* and S. *rimosus*, as well as their mono-cultures, were successively extracted with methanol:dichlormethane (1:1) solution in ultrasonic bath. To determine the significance of their co-cultivation, a range of biological assays were performed on algal and bacterial mono-cultures, as well as co-culture. The antimicrobial activity of extracts against *Staphylococcus aureus* and *Escherichia coli* was tested using the microdilution broth method. Furthermore, the antioxidant activity was investigated by four spectrophotometric methods (reduction of the radical cation (ABTS), the oxygen radical absorbance capacity (ORAC), ferric-reducing antioxidant power (FRAP), the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assays) (Jerković et al. 2021). In terms of anti-aging properties, *in vitro* collagenase inhibition was determined spectrophotometrically according to the manufacturer's instructions. The potential toxicity of tested samples was determined by conducting zebrafish *Danio rerio* embryotoxicity test (OECD 236, 2013).

Results

Co-culture extract showed higher antimicrobial activity (against both *S. aureus* (46.06% of inhibition) and *E. coli* (72.28% of inhibition)) when compared to activities recorded on both tested *C. vulgaris* and *S. rimosus* mono-cultures. The highest antioxidant activity was obtained using the FRAP assay on algal mono-culture and co-cultivation. In terms of anti-aging properties, the highest percent of inhibition of collagenase activity was recorded on *S. rimosus* mono-culture (90.5 %, 88.8 %, 87.8 % of inhibition on 3, 1, 0.5 mg/mL of extract, respectively). Tested extracts in a concentration range from 0.6 to 0.15 mg/mL showed no negative impact on the survival/development of zebrafish *Danio rerio* embryos.

Discussion

As can be seen from the obtained results, co-cultivation of *S. rimosus* and *C. vulgaris* effectively improved bioactive compound synthesis which reflected in higher bioactive potential when compared to the one obtained in mono-cultures. The co-culture extract exerted beneficial effects such as antibacterial, antioxidant and anti-age activities, while at the same time not being toxic. It should be emphasized that observed anti-collagenase activity on *S. rimosus* monoculture and co-cultivation extracts appears to be related to oxytetracycline, which is widely known as Streptomyces product. Collectively, co-cultivation of macroalga *C. vulgaris* and bacteria *S. rimosus* has great commercial potential and opens possibilities for the discovery of novel bioactive molecules.

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SUSTAINABILITY ASSESSMENT OF AQUACULTURE OPTIMISATION OPTIONS IN LAND-BASED SEABASS AND SEABREAM FARMING IN ITALY

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Introduction

Environmental concerns, resource optimisation goals, costs abatement, and market competitivity all make the aquaculture sector not exempt from seeking cheaper and less impacting solutions, be them technological or organisational ones.

Although not possible everywhere, especially in dry areas, land-based fish farming systems promise to allow for resource saving for both management and transportation.

The improvement of this kind of operating systems addressed here seabass and seabream farms, selected in a convenient area of Tuscany, Italy, very close to a natural lagoon. Here, academic and business partners are involved, from the action 'New Technologies, Tools and Strategies for a Sustainable, Resilient and Innovative European Aquaculture (NewTechAqua)', funded within European Union's Horizon 2020 programme.

Their objectives are:
1) Decreasing the environmental loads of land based farming;
2) Increasing profitability by decreasing feeding costs, also applying the emerging framework of "precision fish farming" (Føre et al., 2017), with the aim of supporting operators in optimising feeding practices with respect to growth performances, fish welfare, and - indeed - environmental load.

The environmental performances of the solutions proposed to achieve these objectives are evaluated and here preliminarily presented.

Methods

The environmental performances of the land-based seabass and seabream fish farming plant at hand are appraised through well-oiled Life-Cycle Assessment (LCA), standardised and updated by the International Standard Organisation (Arvanitoyannis, 2008; ISO, 2018). Indicators are available from different calculation choices, including the Ecological Footprint (Wackernagel and Rees, 2004), the Global Warming Potential, the Cumulative Exergy Demand, the Water Footprint (Hoekstra et al., 2011), and the ReCiPe sets (Goedkoop et al., 2009).

Results

The potentials, limits, and margins for improvement of the proposed solutions are presented and discussed, bringing together trade-offs between newly invested resources and newly caused environmental impacts and expectedly saved resources and environmental impacts over time. This kind of information represents a basis for further refining of such solutions.

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RAINBOW TROUT (*Oncorhynchus mykiss*) – A SOURCE OF MULTIFUNCTIONAL BIOACTIVE PEPTIDES AND BY-PRODUCTS USED FOR HETEROTROPHIC AND MIXOTROPHIC GROWTH OF *Galdieria sulpuraria*

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Introduction

The present study as a part of CLIMAQUA project aims at full utilization of seafood resources (particularly, farmed fish) for recovery of both high- and low-value products. Even though seafood raw material as a source of multifunctional peptides has already been reported in scientific literature, extraction of bioactive peptides from farmed fish for non-communicable disease prevention, is currently underexplored. Therefore, regarding the production of high-value ingredients, the current study's aim was to identify new multifunctional peptides isolated from rainbow trout (*Oncorhynchus mykiss*) which can be further used in manufacture of functional foods and nutraceuticals. Regarding low-value applications, recirculation and zero-waste re-utilization of aquaculture and fish processing side-streams (sludge after enzymatic hydrolysis of rainbow trout, fish slam and wastewater obtained from fish processing facility of Hofseth AS) as substrate for cultivation of algae (*Galdieria sulpuraria*) which can further be used in fish feed formulations, was addressed.

Methods and materials

Head-on gutted rainbow trout delivered from a local fish processing plant "Hofseth AS" (Ålesund, Norway), was used as raw material for enzymatic hydrolysis. Hydrolysis was performed in 4-litre bioreactors at 50±2°C, adding 0.05 % papain and 0.05 % (w/w) bromelain enzymes at the Department of Biological Sciences Ålesund of Norwegian University of Science and Technology (NTNU). Sludge was collected from the bottom of the bioreactor after the enzymatic hydrolysis for further use as a substrate for cultivation of algae (*Galdieria sulpuraria*). Fish slam and wastewater were delivered from the fish processing facility of Hofseth AS (Ålesund, Norway), physiochemically characterised at NTNU and sent to Germany along with the sludge for algae cultivation trials. Trout hydrolysate was freeze-dried at the Department of Energy and Process Engineering (NTNU) and sent to the Department of Pharmaceutical Sciences of University of Milano (Italy) for analysis of biological activities.

At NTNU, the obtained trout hydrolysate was subjected to physicochemical assays to assess its quality parameters (proximate analysis, degree of hydrolysis, amount of water-soluble proteins, amino acid composition, total carbonyl content, fat content and colour parameters).

A peptidomic investigation (using HPLC-MS/MS technique) was performed at University of Milano to display chemical composition of the trout hydrolysates and identify peptide sequences which are present in the hydrolysate mixture, as well as proteins to which each peptide belongs to. In addition, direct antioxidant activity of the hydrolysate by using a combination of ABTS, FRAP and DPPH assays, was measured.

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Results and discussion

The study has shown that trout hydrolysate has good quality parameters and exerts a multifunctional bioactivity through the ability of reduction of the oxidative stress induced by H_2O_2 in human intestinal Caco-2 cells, as well as inhibition of ACE and DPP-IV activities, respectively, suggesting antioxidant, hypotensive, and anti-diabetic effects. The results of bioactivity investigation indicated that trout peptide mixture is able to scavenge the ABTS and DPPH radicals, respectively. In addition, it increased the reduction of Fe^{3+} at Fe^{2+}. At cellular levels, the same peptides reduce oxidant stress induced by H_2O_2 in Caco-2 cells. The high presence of hydrophobic peptides within the hydrolysate is correlated to the antioxidant effect and other biological activities.

Regarding low-value applications, the results have shown that fish slam has a potential to be used as a substrate for the algae cultivation. However, more investigation is needed to establish the conditions for re-utilization of sludge and wastewater for the efficient cultivation of *Galdieria sulpuraria*.

Conclusion

The study has shown that peptide mixture of the rainbow hydrolysate can reduce with a dose-response trend both ACE and DPP-IV activities, suggesting hypotensive and anti-diabetic activities of the product. Thus, rainbow hydrolysate can be used as a functional ingredient in the food industry, as well as multifunctional nutraceutical.

Norway has established efficient aquaculture and fish production systems and will work further on methods for full utilization of seafood resources through zero-waste utilisation of fish processing side-streams. The cooperation between Norway and other European aquaculture actors involved in CLIMAQUA project will strengthen regional capabilities and contribute to food security and resource utilisation efficiency under climate change expected within 1.5 or 2°C until 2050.

EFFECT OF*Perkinsus olseni In Vivo* INFECTION ON *Ruditapes decussatus* CONDITIONING AND SPAWNING EFFICIENCY

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Introduction

Worldwide, the major problems that clam's production faces are the lack of constant seed supply and drastic annual fluctuations of seed recruitment in wild beds. Moreover, clam's production/survival has been greatly affected by anthropogenic actions and climate changes (for instance, temperature increase, salinity drops, ocean acidification, etc.). Among the major problems is the lack of resistance to infections by pathogenic organisms such as bacteria, viruses, protista, etc. (Carella et al., 2015) leading to extremely high mortality rates (Gosling, 2008) that can reach 80-90%. This is also applied to grooved carpet shell, *Ruditapes decussatus*, a European native clam species. In the last two decades, *R. decussatus* production has declined mainly due to over-exploration of natural clam beds, pollution, significant temperature and salinity variations and pathologies caused by bacteria, virus and protozoa. In particular, new diseases like Perkinsosis caused by the protozoan parasite *Perkinsus olseni*, associated with environmental degradation, are causing abnormal mortalities (Matias, 2013).

The effects on the reproduction capacity of bivalves under pathological infections have been extensively studied, since it is a major cause of production decrease in natural marine and estuarine beds (Dittman et al., 2001; Villalba et al., 2004). The effect of *Perkinsus olseni* infection on the reproduction ability of clams has been underestimated. Although some studies found evidence of reduction of egg production and delay in gonad maturation after infection, the total effect of the infection is still unclear.

Material and methods

In this study, *Ruditapes decussatus* clams from a naïve population were injected with two different doses of *Perkinsus olseni* parasites, a low infection dose ($5{\cdot}10^3$ parasite cells per clam) and a high infection dose ($5{\cdot}10^5$ parasite cells per clam). Clams were maintained during two months for conditioning and at the end of the experiment the spawning was induced, larvae production was quantified, and mortality was evaluated during their first days of life. Also, the infection level and the oxidative status of animals were evaluated at days 0, 30 and 60 post-injection.

Results

The combined effects of *P. olseni* infection, conditioning temperature and feeding significantly increased mortality. Also, gonad maturation was delayed in infected animals but with a higher effect in the high infected animals. The reproductive capacity of the clams was impaired in both infected groups with a lower quantity of larvae produced per clam and a higher mortality rate in larvae during the first week.

Conclusion

Finally, this study showed that the production of natural beds with high prevalence of *Perkinsus* spp. could be compromised by deregulation of the natural reproduction cycle and decreased larval production by infected animals, probably due to a combination of lower egg production and lower lipid reserves in the infected clams.

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AN ECOSYSTEM MODELLING FRAMEWORK FOR ANALYSIS AND MANAGEMENT OF EUTROPHICATION AND AQUACULTURE IN COASTAL SYSTEMS

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Introduction and Approach

SUCCESS (System for Understanding Carrying Capacity, Ecological, and Social Sustainability) is a soil to sea modelling framework for integrated coastal management (Fig. 1). SUCCESS addresses (i) the partitioning of nutrient and bacterial loading from land; (ii) the circulation of water and water properties in receiving waters (in this case Belfast Lough) and the exchange with the ocean; (iii) aquaculture inside the system (in this case production and environmental effects of mussel aquaculture, including top-down control of primary production); (iv) system biogeochemistry and ecosystem sustainability, in particular with respect to carrying capacity for aquaculture. Each model has a number of uses *per se* and addresses different management challenges—for instance, SWAT is key for source apportionment of drivers and their connection to nutrient pressures—the linkages among models allow the whole framework to be leveraged to provide policy makers with fact-based, actionable information.

T he well-tested EcoWin ecological model (e.g. Ferreira et al, 2008; Bricker et al. 2018, and references therein) was used in the SUCCESS framework to analyse bottom-up (source control) and top-down (bivalve filtration of phytoplankton and detritus) control of eutrophication symptoms in Belfast Lough, Northern Ireland (UK).

The EcoWin.NET model divides Belfast Lough (130 km^2, maximum depth 23 m) into 52 physical compartments (boxes) with two vertical layers at mid-depth (26 boxes for surface waters and 26 for bottom waters), using a 3D formulation where the circulation is resolved by the Delft-3D hydrodynamic model. EcoWin considers 234 boundaries, of which 232 are land boundaries and 2 are ocean boundaries. Of the former, 21 are rivers and 95 are urban assets discharging directly to the lough. Nutrient discharges from the catchment are modelled using SWAT. Bivalve aquaculture in the lough consists of sub-tidal bottom culture of blue mussels. At present, ten mussel farms are active, all within Inner Belfast Lough, and there is a complementary artisanal fishery of wild mussels.

The SUCCESS framework has been applied in a joint project in Northern Ireland (the Living With Water Programme - LWWP) that brings together the water industry (i.e. supply and treatment), environmental regulators, and aquaculture and fisheries research and policy makers, in order to plan for environmentally sustainable, multi-stakeholder development of a large water body, adjacent to a major city with a population of 640,000. To our knowledge, this is the first time that this type of integrated, multi-stakeholder approach has been used to inform investment decisions at the scale of hundreds of millions of euros by applying ecosystem-based management (EBM).

Results and Discussion

Table 1 shows nitrogen load from both diffuse and point sources to Belfast Lough. The main source of sewage input to the lough is the River Lagan, and about half the nitrogen loading comes from diffuse sources (46%), where bottom-up control has significant social and economic costs.

Nutrient inputs to the lough increase primary production and potentially lead to coastal eutrophication, which would result in a degradation of water quality and ecosystem health and impact economic activities and other uses of the lough.

Table 2 shows that bottom-up control of eutrophication by pro-rata reduction of N loads is significant across all the lough but appears stronger in the southern part due to the location of urban discharge points.

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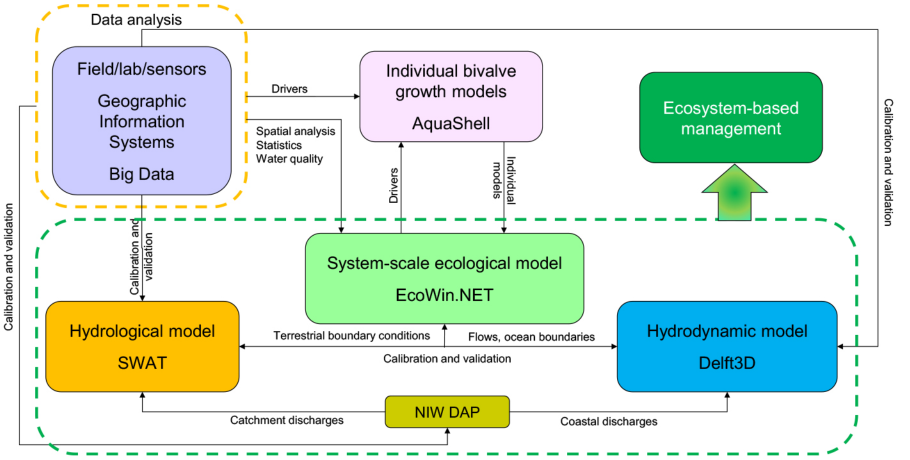

Fig. 1. SUCCESS ecological modelling framework applied to Belfast Lough.

Table 1. Partitioning of nitrogen loads from the catchment to Belfast Lough.

Parameter	**Urban (DAP + septic tanks)**	**Diffuse from land (SWAT)**	**Total**
Nitrogen load (kg y^{-1})	1,411,366	1,204,702	2,616,069
N per unit area (kg ha^{-1})	17.87	15.25	33.11
Population Equivalents (PEQ)	427,687	365,061	792,748
Percentage	54%	46%	100%

Table 2. Effect of 50% nitrogen load reduction on chlorophyll in Belfast Lough.

Chlorophyll P_{90}	**Box 7**	**Box 8**	**Box 11**	**Box 9**	**Box 10**	**Box 20**	**Box 22**	***Box 38***
Standard loading (µg L^{-1})	6.4	14.8	13.3	9.9	10.4	7.3	8.1	13.0
50% loading (µg L^{-1})	4.7	9.8	8.8	7.0	7.1	5.0	5.5	8.6
Difference (%)	-26.6	-33.5	-33.6	-29.9	-32.0	-32.1	-31.8	-33.5

Table 3. Chlorophyll reduction in Belfast Lough with top-down control. From left to right, upper boxes from head to mouth of the lough. Box 31 is the only lower box, included to show differences between upper (Box 5) and lower boxes (Box 31).

Chlorophyll P_{90}	**Box 7**	**Box 5**	***Box 17***	***Box 18***	**Box 24**	**Box 25**	**Box 26**	***Box 31***
Blue mussels (µg L^{-1})	6.4	8.2	5.2	3.9	3.0	0.9	0.8	2.7
No top-down control (µg L^{-1})	9.2	10.9	7.0	5.0	3.5	0.9	0.8	5.1
Difference (%)	-30.7	-25.1	-25.4	-22.2	-13.5	-7.9	-1.0	-47.7

When farmed blue mussels are 'turned off' in EcoWin, chlorophyll decreases significantly in all the lough, particularly in the northern part of the system and close to the mussel farms. Table 3 shows the difference in chlorophyll depending on whether bivalves are active or not and suggests that filtration of phytoplankton and organic detritus makes a significant contribution to top-down eutrophication control. Since 46% of the possible nutrient controls are related to land management (Table 1), and challenging to implement, shellfish aquaculture plays a significant role in meeting water quality standards and Water Framework Directive (WFD) targets.

The SUCCESS modelling framework is a valuable management tool for assessing different scenarios for eutrophication status and legislative compliance, from source control of nutrient emissions to complementary approaches such as shellfish farming for top-down control. If diffuse sources such as agriculture are a significant part of nutrient loading, bivalve aquaculture can play an important role in providing ecosystem services and offset eutrophication symptoms, internalising costs of meeting environmental standards and increasing social capital.

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STABLE ISOTOPE AND FATTY ACID ANALYSIS REVEAL THE ABILITY OF SEA CUCUMBERS TO USE FISH FARM WASTE IN INTEGRATED MULTI-TROPHIC AQUACULTURE

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Introduction

Deposit-feeding sea cucumbers are promising candidates for integrated multi-trophic aquaculture (IMTA), not only for their commercial value, but also for their capacity to process and reduce organic matter in sediment (Cubillo et al., 2016, Chary et al., 2020). However, if fish-sea cucumber IMTA systems are to be developed, there is a need to establish a trophic link between fed and extractive species and to verify the ability of sea cucumbers to assimilate organic matter from aquaculture-derived waste. This study combines carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope and fatty acid (FA) techniques to substantiate the ability of *Holothuria poli* to incorporate dietary organic matter from aquaculture waste in fish-sea cucumber IMTA.

Methods and Materials

This study was carried out between October 2018 and September 2019, at a commercial fish farm (MFF Ltd.), in Malta, central Mediterranean Sea. Sea cucumbers were cultured directly below a gilthead sea bream (*Sparus aurata*) fish cage at 0 m (E0), at 10 m (E10) and 25 m (E25) distances from the cage, based on particulate depositional model outputs (Baltadakis et al., 2020). Reference sites, R1 and R2, were set up over 800 m away from the fish cages. In February, May and September 2019, sea cucumbers were sacrificed from each of the three cages set up at every site to assess the isotopic and FA composition of their body wall tissue. As potential food sources, fish faeces from sea bream and fish feed administered to the farmed fish were sampled for stable isotope and FA analysis. Also, seagrass *Posidonia oceanica* was collected where present at the sites, whereas suspended matter and seafloor sediments were sampled at each site for analysis.

Results

Mass mortalities were recorded at E0 within the first month due to smothering by settled wastes. The sampled food sources at the IMTA and reference sites were characterised by distinctly different isotopic signatures that allowed organic matter pathways to be traced and distinguished. Temporal variation in food availability and quality was reflected in the sea cucumber diet. Still, farm waste (FW) was generally the dominant dietary source for *H. poli* at E10 and E25 (Fig. 1), whereas the isotopic signatures of sea cucumber tissues at reference sites were not explained by the sampled food sources.

FW and sediments near fish cages were characterised by higher abundance of oleic (OA, 18:1*n*-9) and linoleic (LA, 18:2*n*-6) acids, presumably associated with terrestrial vegetable oils in fish feeds, relative to the reference sites. Importantly, fatty acid profiling also revealed a higher abundance of these FAs in sea cucumber tissue at E10 and E25, whereas sea cucumbers at reference sites were characterised by arachidonic acid (20:4*n*-6) acid, EPA (20:5*n*-3) and DHA (22:6*n*-3) instead. These important differences in sea cucumber tissue composition were driven by aquaculture-derived waste near fish cages.

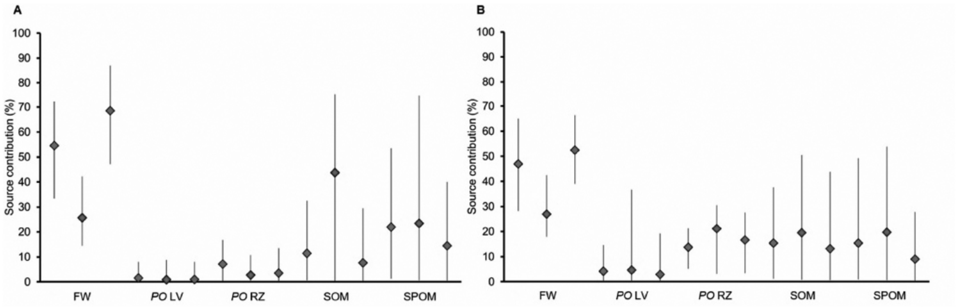

Fig. 1. Organic matter source contribution (median ± 95 credible intervals) to *H. poli* diet during the sampling times, February (in blue), May (in red), and September (in green) in A. E10, and B. E25. FW: farm waste, PO LV: *P. oceanica* leaves; PO RZ: *P. oceanica* rhizomes; SOM: sedimentary organic matter; SPOM: settling particulate organic matter.

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Discussion and Conclusions

Isotopic findings substantiated the ability of holothurians to use FW as a nutritional source (Yokoyama, 2013, Xia et al., 2015). The high relative abundance of OA and LA in sea cucumbers near fish cages, presumably linked to the terrestrial plant oil in feeds, confirms the nutritional benefits of IMTA-produced sea cucumbers. The validated uptake of aquaculture-derived organic waste could create economic value for producers to invest in sustainably farmed products. Moreover, the increasing preferences for eco-labelled aquaculture products present economic opportunities to adopt environmentally friendlier technologies and encourage diversification in the Mediterranean. Complementary evidence revealed the dietary significance of aquaculture-derived organic matter for sea cucumber production near fish cages. Findings validated the trophic link in fish–sea cucumber IMTA and the potential of sea cucumbers as value-added IMTA products.

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STABLE ISOTOPE AND FATTY ACID ANALYSIS REVEAL THE ABILITY OF SEA CUCUMBERS TO USE FISH FARM WASTE IN INTEGRATED MULTI-TROPHIC AQUACULTURE

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Introduction

Deposit-feeding sea cucumbers are promising candidates for integrated multi-trophic aquaculture (IMTA), not only for their commercial value, but also for their capacity to process and reduce organic matter in sediment (Cubillo et al., 2016, Chary et al., 2020). However, if fish-sea cucumber IMTA systems are to be developed, there is a need to establish a trophic link between fed and extractive species and to verify the ability of sea cucumbers to assimilate organic matter from aquaculture-derived waste. This study combines carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope and fatty acid (FA) techniques to substantiate the ability of *Holothuria poli* to incorporate dietary organic matter from aquaculture waste in fish-sea cucumber IMTA.

Methods and Materials

This study was carried out between October 2018 and September 2019, at a commercial fish farm (MFF Ltd.), in Malta, central Mediterranean Sea. Sea cucumbers were cultured directly below a gilthead sea bream (*Sparus aurata*) fish cage at 0 m (E0), at 10 m (E10) and 25 m (E25) distances from the cage, based on particulate depositional model outputs (Baltadakis et al., 2020). Reference sites, R1 and R2, were set up over 800 m away from the fish cages. In February, May and September 2019, sea cucumbers were sacrificed from each of the three cages set up at every site to assess the isotopic and FA composition of their body wall tissue. As potential food sources, fish faeces from sea bream and fish feed administered to the farmed fish were sampled for stable isotope and FA analysis. Also, seagrass *Posidonia oceanica* was collected where present at the sites, whereas suspended matter and seafloor sediments were sampled at each site for analysis.

Results

Mass mortalities were recorded at E0 within the first month due to smothering by settled wastes. The sampled food sources at the IMTA and reference sites were characterised by distinctly different isotopic signatures that allowed organic matter pathways to be traced and distinguished. Temporal variation in food availability and quality was reflected in the sea cucumber diet. Still, farm waste (FW) was generally the dominant dietary source for *H. poli* at E10 and E25 (Fig. 1), whereas the isotopic signatures of sea cucumber tissues at reference sites were not explained by the sampled food sources.

FW and sediments near fish cages were characterised by higher abundance of oleic (OA, 18:1*n*-9) and linoleic (LA, 18:2*n*-6) acids, presumably associated with terrestrial vegetable oils in fish feeds, relative to the reference sites. Importantly, fatty acid profiling also revealed a higher abundance of these FAs in sea cucumber tissue at E10 and E25, whereas sea cucumbers at reference sites were characterised by arachidonic acid (20:4*n*-6) acid, EPA (20:5*n*-3) and DHA (22:6*n*-3) instead. These important differences in sea cucumber tissue composition were driven by aquaculture-derived waste near fish cages.

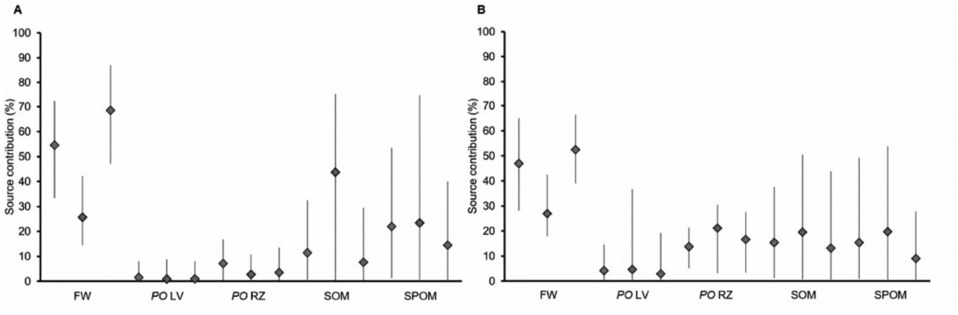

Fig. 1. Organic matter source contribution (median ± 95 credible intervals) to *H. poli* diet during the sampling times, February (in blue), May (in red), and September (in green) in A. E10, and B. E25. FW: farm waste, PO LV: *P. oceanica* leaves; PO RZ: *P. oceanica* rhizomes; SOM: sedimentary organic matter; SPOM: settling particulate organic matter.

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Discussion and Conclusions

Isotopic findings substantiated the ability of holothurians to use FW as a nutritional source (Yokoyama, 2013, Xia et al., 2015). The high relative abundance of OA and LA in sea cucumbers near fish cages, presumably linked to the terrestrial plant oil in feeds, confirms the nutritional benefits of IMTA-produced sea cucumbers. The validated uptake of aquaculture-derived organic waste could create economic value for producers to invest in sustainably farmed products. Moreover, the increasing preferences for eco-labelled aquaculture products present economic opportunities to adopt environmentally friendlier technologies and encourage diversification in the Mediterranean. Complementary evidence revealed the dietary significance of aquaculture-derived organic matter for sea cucumber production near fish cages. Findings validated the trophic link in fish–sea cucumber IMTA and the potential of sea cucumbers as value-added IMTA products.

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IDENTIFYING SUITABLE SETTLEMENT SUBSTRATES AND FEEDS FOR EARLY STAGE GROWTH OF THE SEA URCHIN *Tripneustes gratilla*: IMPLICATIONS FOR HATCHERY PROTOCOLS AND PRODUCTION

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Introduction

Successful larval settlement and metamorphosis has been linked to the recognition of specific substrates or substratum-specific biochemical signals. In many intensive aquaculture systems, the required morphogenesis-inducing substances are often absent. Sea urchin settlement and metamorphosis cues are highly species-specific and may include microalgae, natural surfaces (e.g. porous rocks), adult conspecifics, coralline and other macroalgae, specific chemicals, and bacterial biofilms, particularly those found on the surface of macroalgae. Commercial abalone and urchin farms often use biofilms that develop in the systems naturally for larval settlement (Affan et al., 2015); but this method is often highly inconsistent and seasonal and largely considered ineffective for *Tripneustes gratilla* (<2% settlement, with poor post-settlement survival) (Mos et al., 2011). Certain cues may well induce settlement but not necessarily sustain growth and ensure survival of larvae during the post-settlement phase (Mos *et al.,* 2011), because the settlement substrate is either indigestible or nutritionally poor. *T. gratilla* requires a diet capable of promoting high survival and growth during these fundamental stages of development. This study investigated settlement success, post-settlement survival and growth of *T. gratilla* during different periods of the juvenile phase fed a variety of natural and artificially created substrates/diets.

Materials and Methods

Nine settlement substrates were tested, including natural substrates of *Ulvella lens*, fresh *Ulva* and *Nitzschia,* as well as artificially created substrates using alginate with the addition of either dried *Ulva*, dried *Isochrysis galbana*, a probiotic *Vibrio midae* SY9, a combination of *V. midae* SY9 & an *Ulva* exctract, ethanol solvent (control) or no additive (control). Competent larvae were settled directly into small troughs (L:200×W:105×D:45mm) contained within a flow-through experimental system. Each treatment had 4 replicates within randomly assigned tanks. Competent larvae (n=35) were placed in each trough and the number of larvae to successfully completed metamorphosis within a 60h period was recorded. Successfully settled larvae were maintained in their respective treatments for a period of 4 weeks to assess post-settlement growth (by measuring test diameter of 10 random individuals) and survival, which was recorded weekly for each treatment using a Nikon SMZ1500 stereomicroscope. Substrates were replaced once a week when the individual troughs were cleaned. After 4 weeks, urchins from the best performing treatment (*U. lens*) were used to assess the effects of weaning urchins from post-larval feeds onto the macroalga *Ulva*. Urchins from the latter treatment were randomly divided into 2 treatments of 4 replicates each. One treatment continued to receive *U. lens* for three weeks before being fed fresh *Ulva* for an additional 3 weeks, whereas the second treatment group was offered fresh *Ulva* for the entire 6 week period.

Results and Discussion

Settlement success varied greatly between substrates. The 3 best performing treatments, not significantly different from each other, were *Ulva*, *Ulvella lens* and *Nitzschia sp.*; which induced 67, 62 and 41% larval settlement, respectively. Settlement success was less than 30% for the remaining treatments, with no significant differences between them. Survival of larvae settled and maintained on the *U. lens* substrate by the end of the 4 week growth trial was significantly higher (61%) compared with all other treatments, which had an average survival < 20%.

Juvenile urchin test diameter (TD) at week 4 was significantly greater in the *U. lens* treatment (3507μm), compared with all other treatments that had an average TD of 508μm, with no significant differences between the latter treatments. Thus, diets other than *U. lens* do not appear to be suitable post-settlement diets for *T. gratilla*, even though both *Ulva* and *Nitzschia* induce high settlement. Following the dietary change at week 4, urchins maintained on *Ulvella* were significantly larger at week 7 (6874μm; Fig 1) compared to juveniles fed fresh *Ulva* (5260μm). After 7 weeks both treatments were offered only *Ulva*, but the difference in TD did not change between the 2 treatments and urchins maintained for 7 weeks on *Ulvella* remained significantly larger at the end of week 10 (14278μm TD), compared with juveniles transferred to a diet of fresh Ulva three weeks earlier (11638μm TD).

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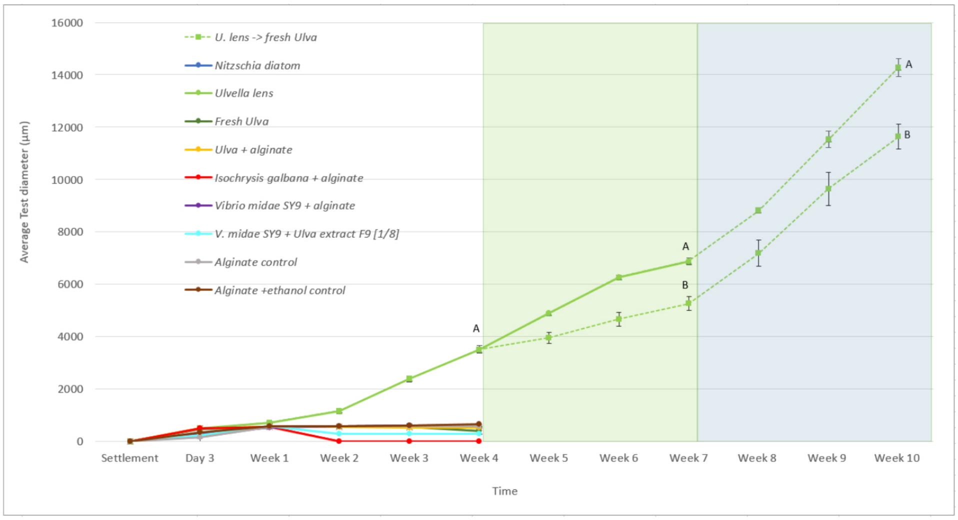

Figure 1: Average test diameter of juvenile *T. gratilla* over a 10 week period post settlement fed a variety of natural and artificial diets. Shaded areas represent dietary changes during weaning, from Ulvella to Ulva as indicated in the figure legend.

Our study suggests *Ulvella* is a superior substrate for *T. gratilla* larval production, capable of producing juvenile sea urchins of ca. 1.5cm in just 10 weeks post-settlement. These findings have important implications for the commercial production of this high value species, highlighting the importance of suitable substrates for both settlement and post-settlement feeding. The study further shows that the timing of weaning juveniles from post-settlement diets to adult feeds can have significant effects on growth and potential effects on overall production times, which could have significant effects on timelines for the production on urchins for harvest.

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EFFECT OF COLD PLASMA TREATMENT ON THE SHELF-LIFE OF SEA BREAM FILLETS

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Introduction

Cold plasma is an emerging sanitizing technology that has been implemented in recent years to extend the shelf-life of food product (Andoni *et al*., 2022.) (Olatunde *et al*., 2021). Seafood products are among the main constituents of food diets, important for their content of vitamins, proteins and other essential constituents such as polyunsaturated fatty acids (PUFA) (FAO, 2020). The main concern associated with this matrix is the rapid deterioration of freshness and quality. The aim of this study was to evaluate the application of cold gas plasma (CP) as a non-thermal preservation technology to prolong the shelf life of sea bream *(Sparus aurata)* fillets, stored in modified atmosphere packaging (MAP).

Materials and methods

The samples were divided into three experimental groups, two of which were respectively treated in air (18 kV for 20 min), or argon (80%) and oxygen (20%) mixture (18 kV for 20 min), while the third one was not treated (control). A plasma source with an SDBD (surface dielectric barrier discharge) configuration was used for plasma generation. The samples were following packed in conventional MAP (80% N_2 and 20% CO_2), and subjected to refrigerated storage (4±1 °C) for 14 days. During this period, at different storage time, possible differences among the samples were investigated in terms of quality characteristics (e.g. O_2 and CO_2 in the package headspace, pH, water content, t-Bars, texture, colour and sensorial properties, microbes load). Experimental data were subjected to two-way analysis of variance (ANOVA) to determine the significant differences among samples during the storage and in each sampling time. Tukey HSD (Honestly Significant Difference) multiple range test, at a significance level of $p < 0.05$ was applied.

Results

Considering the physico-chemical characteristics, with the application of cold air plasma for 20 min, a reduced pH value of the product was recorded during storage, probably caused by the formation of acids (HNO_3, HNO_2, H_2O_2 and O_3) in the aqueous phase of the fish flesh, and a simultaneous slowing down of the natural increase in pH during shelf-life, promoted by the reduction of enzymatic activities. All the samples did not show significant differences in terms of texture parameters, evidencing that the applied treatments did not promote protein oxidation and/or evident structural modification, This aspect was also confirmed by the constant maintenance of the water content during storage for all the considered samples.

The major limitation of the application of cold plasma to fish matrices, or those characterized by a high proportion of unsaturated fatty acids, can be lipid oxidation, considering the 'oxidative nature' of this technology for food applications. Actually cold plasma is constituted by various reactive species, including free radicals, positive and negative ions. These species responsible of its potential sanitizing effect can also stimulate the oxidation process in food products with a high fat content, having a negative impact on their sensory and nutritional quality.

The present study showed that already by the sixth day of storage, the samples treated by argon plasma, reached the quality limit of t-Bars > 4 mg MDA/kg of fresh product compared to the samples treated by air plasma (2.4mg MDA/kg) and to control samples (1.20 mg MDA/kg). Color measurement following the application of both treatments also revealed significant changes in the red-green index colour (a*), the reduction of which can be correlated with lipid oxidation of the matrix.

The microbiological analyses conducted were evaluated for an extended shelf life (20 days). The presence of total mesophiles was evaluated, setting a limit of 6 Log_{10} CFU/g. Results showed that especially at day 10 and day 20 of storage, the treated samples had a lower total load than the control; and in particular at day 10, the control and argon group exceeded the set limit, while the data obtained from the analysis of the air-treated samples showed that the value was still below the limit. At day 20, the differences were significant, but all experimental groups were considered to be microbiologically unacceptable.

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Although the sensory analysis showed that the overall visual and olfactory acceptability by the consumer did not result in significant variations, this technology seemed to accelerate oxidative processes. For this reason, it could be useful to conduct further studies to investigate the application of the 'hurdles theory', which envisages the application of cold plasma in conjunction with other stabilizing factors, such as the use of antioxidants etc., in order to minimize the negative side effects on the food matrix of the CP treatment (e.g. lipid oxidation).

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USE OF PULSED ELECTRIC FIELDS FOR MODULATING MASS TRANSFER DURING SALTING OF SALMON FILLETS

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Introduction

Salting is one of the oldest and simplest methods for preserving large quantities of fish for long periods of time. It is often used by the industry in combination and/or as a pre-treatment to other traditional heat/mass transfer processing techniques (e.g. smoking, drying and cooking) (Birkeland and Bjerkeng, 2005).(De Jong *et al*., 2016) This processing step results in safer products with a better sensory appearance, but the salting kinetics require long times for the salt to diffuse into the product and to work properly. Among existing emerging technologies, pulsed electric field (PEF) is a non-thermal treatment that has been shown to be effective in increasing mass transfer to both plant and animal tissues, minimally affecting the nutritional value, flavour, colour and texture of the products (Sigurjon *et al*. 2014). The aim of the present study was to apply PEF to atlantic salmon tissue samples as a pre-treatment to dry salting, to test whether this technique could speed up processing time and achieve higher yields.

Materials and methods

For this study, salmon (*Salmo salar*) fillets purchased from a local supermarket were used, from which 90 samples of size 4 x 4 x 2 cm were obtained from Norwegian and Scottish cuts. The samples were divided into 10 groups of 9 replicates each. The experimental design included 5 sample groups for each salting time (3 and 6 hours). The experimental groups were control (NT) and 4 types of differently PEF pre-treated samples (PEF1, PEF2, PEF3, PEF 4). At the end of the salting times, the samples were rinsed in running water, dried and then subjected to the following analytical determinations: weight change, water activity, NaCl content change, water content change, texture, colour and the level of thiobarbituric acid reactive substances (tBars). The data were analyzed by performing an analysis of variance with one-way ANOVA. Afterwards, the Tukey HSD post-hoc test (with $p < 0.05$) was applied on the averages to reveal the presence of significant differences between the various experimental groups.

Results

The investigations conducted within the framework of this study have shown that pulsed electric fields of 0.64 kV/cm (PEF3), applied prior to the three hours of salmon salting, promoted the salt diffusion into the tissues, leading to increased NaCl retention by the muscle, probably thanks to the PEF permeabilization effect on cell membranes. These process parameters in fact generated a reversible electroporation capable of favoring a more homogeneous distribution of salt within the product, also allowing for a lower percentage of weight variation compared to untreated samples. PEF did not provide any advantages in terms of a faster reduction of the water activity of the samples, especially during the shortest salting times, but it did improve the water retention properties of the salmon with three hour salting, probably because of a more permeable structure that allowed retention of more liquid within the tissue. It is also possible that the results obtained were the consequence of a conformational change in the proteins induced by the applied treatment, which allowed greater NaCl absorption and less water loss from the samples. As far as texture, color and lipid oxidation was concerned, the treatment did not provide any difference in the treated samples compared to the control.

The result obtained may be of great importance to the salmon processing industry because the achievement of higher salt levels, in a product that simultaneously lost less water, can provide an important technological advantage. In fact, the salting process is thus more efficient as processing times are significantly reduced (to only three hours) and higher processing yields are achieved with attractive cost savings for companies, improving the performance of industrial processes.

However, further studies are needed in order to better understand the physico-chemical and biological mechanisms responsible of the obtained results, as a function of the process parameters applied, and to verify the impact of PEF treatment on the most important product quality parameters, such as texture, color and sensorial properties.

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MULTI-TRAIT APPROACH FOR UTILIZING MULTI-GENERATION DATA FOR GENOMIC PREDICTION IN ATLANTIC SALMON

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Introduction

Genomic selection (GS) models in aquaculture breeding schemes are often calibrated using single generation data. As data accumulate over time, however, it might be beneficial to use multi-generation data for training models to improve prediction accuracy. However, it is not clear how to utilize the information as difference in allele frequency, linkage disequilibrium and data structure across generations may limit prediction accuracy. The aim of this study was to examine the potential of implementing multi-trait approach to utilize multi-generation data for genomic prediction and compared it with alternative calibration strategies.

Materials and Methods

Phenotype and genotype data from two generations (i.e., YC2019 and YC2015) of MOWI commercial population were available. Body weight (BW) measurements of 2,670 individuals from YC2019 and of 3,546 from YC2015 were used for this analysis. All individuals were genotyped with the customized NOFSAL3 ~55k SNP.

Four calibration strategies were compared: ***Scenario 1***: *within generation prediction*, where breeding values of YC2019 were predicted using data only form YC2019. S***cenario 2****: Across generation prediction*, where breeding values of YC2019 were predicted using data only from generation YC2015; ***Scenario 3***: *Combined prediction*, where breeding values of YC2019 were predicted with combine phenotype and genotype data of YC2019 and YC2015, and ***Scenario 4:*** *multi-trait prediction*, where breeding values of YC2019 were predicted through fitting YC2019 and YC2015 in a multi-trait model assuming they are two different traits.

The performance of these calibration strategies was tested using Best Linear Unbiased Predictions based on pedigree relationships (PBLUP) and genomic relationships (GBLUP). Variance components and breeding values were estimated using BLUPF90 (Misztal et al., 2018) by fitting single and multi-trait mixed linear models. The single trait model was:

where is a vector of phenotypes, is a vector of fixed effect of the sex (2 levels) and year-class (2 levels, if exit), is a vector of breeding values, is a vector of random residual effects and are incidence matrices. It is assumed that and , where are genomic, and pedigree-based relationship matrices respectively, is additive genetic variance and is random residual variance. The multi-trait model was:

Where all the model components are as defined previously, except in a multi-trait model structure.

Prediction accuracy and the bias of the prediction models were assessed based on 30 replications of a cross-validation scheme. In the cross-validation, the phenotypes of 15% of individuals from each YC2019 family were masked and their breeding values were estimated using the genotypes and phenotypes of the reference population according to the calibration scenarios. The accuracy was computed as the correlation between the estimated breeding value (pedigree/genomic, PEBV/GEBV) with the phenotype and scaled by the square root of heritability of the BW for YC2019. The bias was computed as the regression coefficient of phenotypes on either PEBV or GEBV. The means and standard errors for the prediction accuracy and biases were calculated from the 30 replications.

Results and Discussion

Estimates of heritabilities and genetic correlation using single and multi-trait models are summarized in Table 1. The heritability estimates were moderate to high (0.28 - 0.46) and were within the range of what have been reported for BW (e.g., Tsai, et al., 2015), however, the heritability estimates form multi-trait model were higher for both generations. The estimated genetic correlation between YC2015 and YC2019 was 0.72 (Table 1).

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$$y = X\beta + Zu + e$$

where y is a vector of phenotypes, β is a vector of fixed effect of the sex (2 levels) and year-class (2 levels, if exit), u is a vector of breeding values, e is a vector of random residual effects X and Z are incidence matrices. It is assumed that $u \sim N(0, G/A\,\sigma_u^2)$ and $e \sim N(0, I\sigma_e^2)$, where $G\ and\ A$ are genomic, and pedigree-based relationship matrices respectively, σ_u^2 is additive genetic variance and σ_e^2 is random residual variance. The multi-trait model was:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

Where all the model components are as defined previously, except in a multi-trait model structure.

Dataset	Single trait	Multiple traits	
		YC2015	YC2019
YC2015	0.287(0.032)	0.336 (0.025)	
YC2019	0.277 (0.032)	0.716 (0.046)	0.464 (0.032)

Table 1: Heritabilities and genetic correlation estimates for BW for YC2015 and YC2019

Prediction accuracies and bias for the different calibration scenarios are summarized in Table 2. The prediction accuracies found in this study are within the range of reported estimates for both pedigree and genomic datasets. The highest prediction accuracy was obtained when multi-trait model is used to combine the two generations of data (Table 2). Merging YC2015 and YC2019 data did not improve prediction accuracy compared to only using YC2019 data and resulted in slightly deflated estimates.

Table 2: Genomic prediction and bias for body weight in YC2019 estimated using different calibration strategies

Scenarios	**GBLUP**		**PBLUP**	
	Accuracy	**Bias**	**Accuracy**	**Bias**
YC2019 predicted with **YC2019**	0.83±0.08	1.10±0.12	0.62±0.06	0.99±0.12
YC2019 predicted with **YC2015**	0.15±0.12	0.78±0.27	0.28±0.09	0.54±0.17
YC2019 predicted using combined **YC2015** and **YC2019**	0.82±0.07	0.92±0.08	0.60±0.07	0.82±0.13
YC2019 predicted with **multi-trait** approach of **YC2015** and **YC2019**	0.88±0.07	1.01±0.10	0.63±0.06	0.63±0.06

These results demonstrate that the use of existing multi-generation data with appropriate modeling can improve prediction accuracy and could enhance cost-effectiveness of GS in practical aquaculture breeding. It also concluded that phenotyping at each generation if vital to maintain prediction accuracies.

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ADAPTIVE EVOLUTION FOR OPTIMIZATION OF AN INDUSTRIALLY-RELEVANT MICROALGA

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Introduction

Fish oil is currently the main source of omega-3-polyunsaturated fatty acids (ω-3-PUFAs) for the increasingly growing aquaculture feed market, and the Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) global consumer markets [1]. About 1/3 of wild fish stocks are currently overfished, and the remainder of the wild fish stocks are already exploited at maximal sustainable levels [2]. This alarming decrease in wild fish stocks caused by unregulated fishing and its deleterious effect on marine ecosystem, as well as the issue of potential bioaccumulation of toxic pollutants in fish oil, have recently promoted research on alternative sources of these valuable fatty acids [1] . Microalgae are thought to be the primary producers of ω-3-PUFAs in the marine food chain and could therefore serve as a direct source of these nutrients for human and animal use [3].

Thraustochytrids are heterotrophic unicellular marine protists, very well known for their ability to accumulate a high content of ω-3-PUFAs. What makes thraustochytrids advantageous over other oleaginous unicellular microorganisms is, besides accumulation of a high PUFA content, their competency for the industrial scale fermentations due to their fast heterotrophic growth and their production of toxins-free oils [4]. They accumulate lipids in the cell as a defence mechanism against various stress factors, and therefore once triggered they activate their energy reserves and convert them into high concentrations of fatty acids [5]often considered as non-photosynthetic microalgae. They have been isolated from a wide range of habitats including deep sea, but are mostly present in waters rich in sediments and organic materials. They are abundant in mangrove forests where they are major colonizers, feeding on decaying leaves and initiating the mangrove food web. Discovered 80 years ago, they have recently attracted considerable attention due to their biotechnological potential. This interest arises from their fast growth, their specific lipid metabolism and the improvement of the genetic tools and transformation techniques. These organisms are particularly rich in ω3-docosahexaenoic acid (DHA. Thraustochytrids can produce biomass with more than 50% of their cell dry weight as lipids, in which ω-3-PUFAs account for more than 40% [4]. Considering the lack of genomic insights and detailed studies on lipid biosynthesis in thraustochytrids, as well as the complexity of stress adaptation, rational genetic engineering of these microorganisms is limited. Also, oils with high PUFA content and other valuable metabolites produced by genetically modified microalgae are still not accepted by the food and pharmaceutical industries in many countries. Therefore, Adaptive Laboratory Evolution (ALE) serves as an effective tool to study the molecular-level response of microorganisms to stress and adaptation and can aid in designing genetic engineering strategies for optimizing microbial production systems and constructing new microalgal strains [6] DNA sequencing, bioinformatics, and genetic engineering. Accordingly, Adaptive Laboratory Evolution (ALE. In this study, a combined two-stage ALE approach is proposed to obtain a non-transgenic mutant with a potentially higher cell growth/biomass production and stable lipid production (Figure 1).

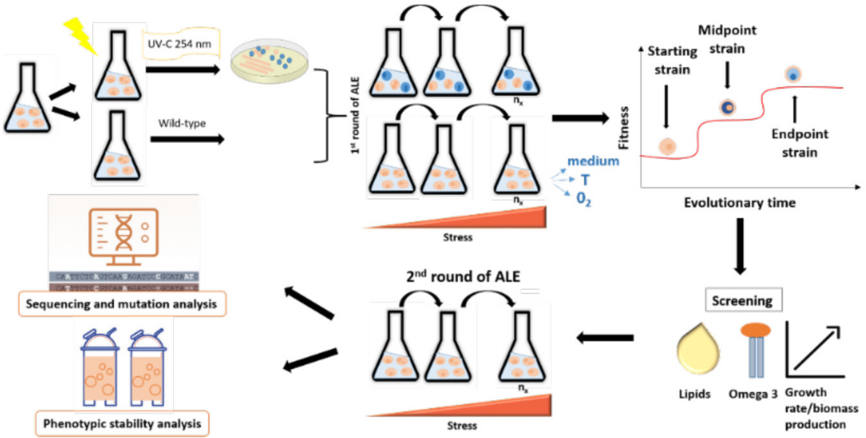

Figure 1. Schematic of a proposed two-phase ALE.

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Aim

To enhance the phenotype of a thraustochytrid strain using ALE, and to identify the possible molecular mechanisms underlying such an adaptation.

Methodology

Pre-screening and optimization of culture conditions

Various experimental cultivation systems and conditions of propagation were tested, in order to establish the starting stress parameters for ALE. Optical density was determined by measuring absorbance at 680 nm, cell density using cell counts via a hemocytometer and cell dry weight was determined gravimetrically. All of these parameters were measured daily in order to determine the cell growth curves. Lipid quantification using fluorescent dye BODIPY 505/515 was tested and will be further optimized for use in screening.

De novo whole genome sequencing and assembly

Long-read Nanopore MinION sequencing was used for the whole genome sequencing in conjunction with Illumina NovaSeq 2x150 bp sequencing. Final de novo genome assembly was conducted using a hybrid approach combining high depth Illumina short reads with Nanopore long reads.

Results and Discussion

A two-stage ALE approach was designed and starting stress parameters were determined. Initial pre-screening experiments set a baseline for future comparison with the resulting mutant strains. The Nanopore MinION sequencing run produced around 20.3 Gb of data, altogether generating an approximate 450x coverage of the genome. Around 2.5 M reads were generated with an N50 value of up to 11.7 kb. An additional 6.8 Gb of paired-end 2x150 bp Illumina reads were generated, representing approximate genome coverage of 150x. The final Illumina-polished assembly generated with Flye showed improved quality and completeness with >95% sequence identity, 91.09% of complete BUSCO genes and greatly reduced indels to 26.10 per 100 kbp.

Conclusions

A high-quality genome assembly was generated that will serve as a reference for the subsequent mutant analysis. ALE experiments coupled with further omics-based analysis including transcriptomics, proteomics and metabolomics will allow for deciphering the connection between genotype and phenotype in the evolved strain compared to the original strain.

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UNDERSTANDING THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) GROUP LEVEL FEEDING BEHAVIOUR IN AQUACULTURE PONDS

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Introduction

In shrimp aquaculture, feed can comprise up to half of total production costs (Engle *et al.* 2017). However, little is still known about shrimp reactions towards feeders, and it is unknown whether shrimp exhibit anticipatory behaviour. Large swarms (or troops) of shrimp were previously reported from SCUBA observations in ponds (McNeil 2001), but observations on the shape of swarms, size distribution, movements and triggers of such formations are yet to be published in penaeid shrimp under commercial conditions. Furthermore, it is worth noting that only a fraction of the pond is fed, irrespective of the feeding strategy. What happens to the unfed areas of the pond is yet to be investigated. The objectives of this study were therefore to understand the effects of feed distribution events, time of day and displacement of the feeding area on shrimp behaviour in a semi-industrial shrimp pond.

Materials and methods

The trial was repeated three times in the same pond (7 x 4 m, length x width) over a period ranging from July to October 2021 in Zhuhai, China; each trial lasted 6 days after an initial acclimation period of 5 days. Three cameras were positioned approximately 1.5 m apart at the bottom of the pond and feed was provided through a PVC pipe either below camera 1 or 3 corresponding to the smaller edges of the pond (with camera 2 in the middle). Feeding was performed 6 times a day, and the feeding area was moved between cameras 1 and 3 every morning. Each observation session with the cameras lasted 20 minutes ranging from 10 minutes before feed was provided to 10 minutes after distribution. Footage was then analyzed for shrimp identification and tracking using a customized software based on the image instantiation algorithm YOLACT highly optimized for this task. This deep neural network was previously trained with a comprehensive and manually labelled dataset comprising of frames of shrimp in the pond. When water conditions were too challenging or the number of shrimps too high for the software to reliably identify and track the animals, shrimp coordinates were manually obtained through EthoVision XT's manual tracking feature.

Results

The main results include a significant effect of camera location and its interaction with time around feed distribution events on the average observed density in individuals. A gradient in the density in individuals appeared just after feed dispersal, with the highest observed density around the feeding area and the lowest on the opposite side of the pond. A significant effect of feed distribution events was also found on the average individual speed, with shrimp being more active during and after feed dispersal than before. Time of day also had a significant effect on the average nearest neighbour distances, with shrimp staying further apart from each other in the observed areas in the evening (i.e. 7 pm) compared to the rest of the day (i.e. 7.30 am and noon).

Discussion

These preliminary results show that feed distribution events induce large movements in shrimp ponds, and not only around the feeding area. Further analyses (ongoing) will provide more information on how those movements are structured, investigating other metrics such as shrimp orientation, swimming direction and polarity, and shrimp size distribution around the feeding area. From previous observations (McNeil 2001), we predict that compact troops or swarms will be led by the largest individuals, with swimming directions oriented towards the feeding area likely observed immediately after feed dispersal.

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INFLUENCE OF TURBIDITY ON GROUP LEVEL RESPONSES TO FEEDING IN THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Introduction

Our understanding of individual and group behaviour of penaeid shrimps, encompassing several commercially important species such as *Litopenaeus vannamei*, has grown over the past few years (see Bardera *et al*., 2019 for a review). However, most studies investigating shrimp behaviour have used clear water conditions allowing for easier observation. Little is known about the influence of water turbidity on the feeding behaviour and social interactions of penaeid shrimps even though they are commonly reared in highly turbid ponds. Therefore, the aim of this study was to provide a better understanding of the influence of turbidity on *L. vannamei* behaviour. The impact of three water turbidity treatments from suspension of kaolin clay (2.4 ± 0.4, 13.6 ± 0.4 and 30.4 ± 0.7 NTU, Nephelometric Turbidity Unit, mean ± S.E.) on juvenile shrimp group behaviour was investigated, exploring changes in key behaviours related to shrimp interaction with feed and use of the tank environment.

Materials and methods

Litopenaeus vannamei shrimp (6.42 ± 0.28 g; n=96; mean ± S.E., body mass) were used in this study, held inside two 300 L recirculation systems. After an initial acclimation period, shrimp were selected to form 24 groups of four individuals each. Each group of four shrimp was exposed sequentially to three turbidity treatments, with randomized treatment orders. The three turbidities were: clear water (CW, 2.4 ± 0.4 NTU, mean ± S.E.), medium turbidity (MT, 13.6 ± 0.4 NTU) and high turbidity (HT, 30.4 ± 0.7 NTU).

A circular experimental arena was used to observe the behaviour of the groups of shrimps, filled with 50 L of artificial sea water. Light conditions above the experimental arena were the same between trials with a light source directed away from the surface of the tank to avoid light reflection and loss of shrimp tracking. For each recording, shrimp were placed into the experimental arena and after an acclimation period of 10 min, 20 min of footage was recorded using a camera positioned above the water surface providing a top-bottom view of the tank. A known amount of feed (2 g) was added in the centre of the tank before shrimp were initially introduced. Footage was analysed automatically using EthoVision XT version 14 (Noldus Information Technology, the Netherlands) with EthoVision Social Interaction Module. Manual corrections to tracking results were applied, when necessary, particularly when occlusions occurred as individuals crossed paths or remained on the feed. Behaviours displayed by all individuals during each video were determined. To process video footage, an ethogram was developed based on known feeding responses, interactions with the test arena and social interactions.

Results

The main results included a significant effect of turbidity on the average velocity with shrimp at medium turbidity moving faster than at higher turbidity (Darodes de Tailly *et al*., 2022). Another significant effect of water turbidity was observed on the average distance between subjects with shrimp held at high turbidity being closer to each other than in clear water and medium turbidity (Darodes de Tailly *et al*., 2022). There was also a significant effect on the percentage of feed consumed with shrimp held at the highest turbidity ingesting more feed than those observed in clear water. No significant effects of turbidity were found for metrics associated with feeding behaviour (Fig. 1).

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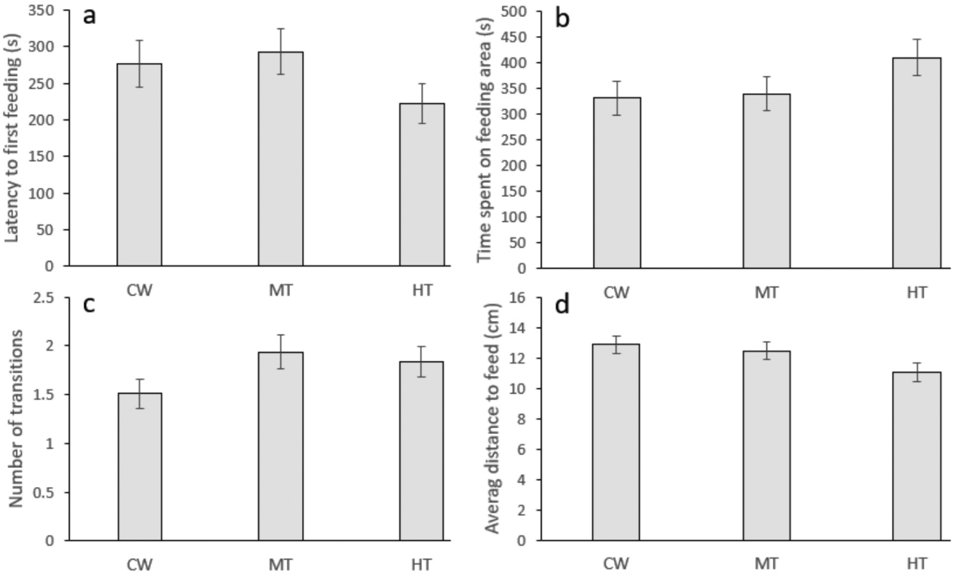

Figure 1: Latency to first feeding (a), time spent on feeding area (b), number of transitions (c) and average distance to feed (d) according to turbidity (clear water CW, medium turbidity MT and high turbidity HT) (n=4 groups for each turbidity sequence, six sequences). Results are presented as means ± S.E.

Discussion

Behavioural differences in shrimp held at different turbidities such as changes in velocity and distance between subjects may be a result of stress responses in reaction to turbidity. The significant increase in feed consumption at high turbidity compared to the clear water treatment aligns with previous observations made by You *et al*. (2006) who found that penaeid shrimp rely mostly on olfaction to detect feed and not vision. The present results therefore highlight the importance of accounting for water turbidity in the behaviour and feed intake of penaeid shrimps, and changing the turbidity conditions of a pond or tank may have a significant impact on production costs for the farmer as a result. Further studies will need to focus on the effect of water turbidity on stress in shrimp, through a combination of behavioural and physiological approaches and the use of other agents to artificially increase turbidity.

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INVESTIGATING THE POTENTIAL OF HYBRID ULTRASOUND - MICROWAVE TECHNOLOGY IN GREEN EXTRACTION OF PHYTOCHEMICALS FROM FRESH BROWN MACROALGAE *Alaria esculenta*

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Introduction

Macroalgae are increasingly being explored as a prospective sustainable food resource and a rich store of numerous bioactive compounds for use in the nutraceutical, pharmaceutical, feed, fuel and cosmetic industries. *Alaria esculenta* is a widely cultivated brown macroalgae species off the Irish coast. Significant research that is being carried out on extraction of phytonutrients from macroalgae, mainly focus on extracting these compounds from the dried and milled biomass due to their higher shelf life, stability, greater uniformity, and ease of transportation and handling compared to minimally processed macroalgae. However, the use of fresh macroalgae can be beneficial, reducing the cost and time of drying as well as reducing the time needed for re-hydration in advance of the extraction processes. This study focused on employing a hybrid ultrasound - microwave (UM) technology to extract total sugars, polyphenols and soluble protein from fresh chopped *A. esculenta* using water as a green extraction solvent.

Materials and methods

Extraction using the integrated UM equipment E200 (IDCO, France) as seen in Fig.1, investigated a set of 27 UM combinations of machine output power of ultrasound (U) ranging from 25-100 W, that of microwave ranging from 336-1340 W for time durations of 5, 10 and 20 min. These treatments were carried out at a fixed macroalgae: water ratio of 1:10. Control runs included using only ultrasound (U), and only microwave (M) at the same output power ranges. After the application of the three types of treatments, the extracts were tested for their total soluble sugar content through phenol sulphuric acid assay, total protein using the bicinchoninic acid assay (BCA), total polyphenol content using Folin–Ciocalteu reagent method and antioxidant activities through DPPH scavenging activity and ferric reducing antioxidant power (FRAP).

Results

Use of U treatment (ultrasound power: 200 W and extraction time: 20 min) yielded the highest amount of total soluble sugars (32.68 ± 2.9 mg glucose equivalents per 100 mg dried extract). UM conditions (ultrasound power: 50 W; microwave power: 1340 W; extraction time: 10 min) recorded maximum total polyphenol content (2.07 ± 0.04 mg gallic acid equivalents/ 100 mg extract) and highest FRAP (38.36 ± 3.36 μM trolox equivalents/ mg dried extract). UM conditions (ultrasound power: 50 W; microwave power: 670 W; extraction time: 5 min) resulted in extracts with the highest DPPH scavenging activity (92.38 ± 0.44 % per mg dried extract). The highest protein content in the extracts (15.62 ± 1.69 mg bovine serum albumin equivalents/100 mg extract) was achieved using UM (ultrasound power: 100 W; microwave power: 1340 W; extraction time: 20 min).

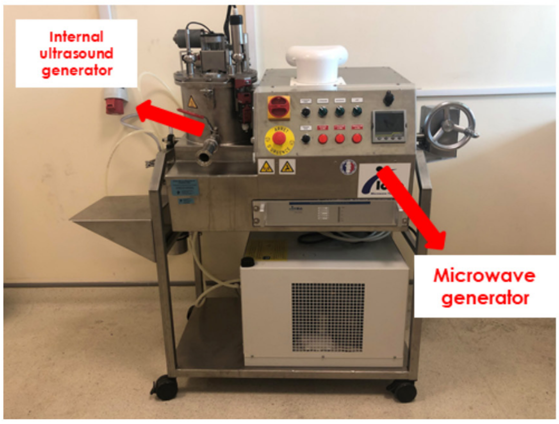

Fig 1: Integrated ultrasound and microwave equipment E200 used in this study.

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Discussion

Combining ultrasound and microwave could harness the dual benefits of each of the individual technologies. Ultrasound via its sono-chemical and sono-physical effects induced through acoustic cavitation helps in disrupting the cell wall of the macroalgae (Chemat et al., 2017) and microwave through dipole rotation and ionic conduction (Moret, Conchione, Srbinovska, & Lucci, 2019) help in increasing the diffusional mass transfer rate and thereby facilitate the penetration of the solvents into the cell, facilitating in multiple ways the extraction of bioactives. This was observed in this study, as UM aided in the maximum extraction of most phytochemicals.

Conclusion

The results of this study indicate that the use of ultrasound alone and in combination with microwave can be employed as green aqueous extraction techniques for extraction of phytochemicals and antioxidants from fresh *A. esculenta*. Further studies will be necessary in order to establish the influence of different extraction forces on individual constituents of macroalgae.

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EXTENDED CO-FEEDING WITH *Artemia* sp. OR ROTIFERS PROMOTES INTER-INDIVIDUAL HOMOGENEITY AND ENHANCE GROWTH PERFORMANCE OF ZEBRAFISH LARVAE

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Introduction

Zebrafish is a model species used for biochemical research and as a model for aquaculture nutrition and aquactic toxicology (1). Moreover, zebrafish presents numerous technical advantages as a model species such as well-established target mutagenesis technologies, embryo transparency, rapid growth and high fecundity (2,3)but their nutrition and housing requirements continue to elude researchers. Diet and housing density were predicted to affect weight change and reproductive success in 120 days postfertilization (dpf. Due to those multiple advantages, the use of zebrafish as a model is increasing worldwide in the past decades. Zebrafish larvae are traditionally reared with live preys such as rotifers and *Artemia* sp., microdiets or using both strategies in combination in a variety of feeding protocols. At the moment, there is still a lack of consent among the zebrafish community on the establishment of a common feeding protocol. This lack of standardization results in differences in the fish weight, embryo production, and it can also impact on the age of sexual maturity. Additionaly to the the biological impacts, the lack of standardized diets also compromises the replicability of results.

Therefore, this work aimed to investigate the effects of the most used feeding protocolos to promote an adequate development and survival.

Materials and methods

Zebrafish embryos (AB wild-type strain) were obtained from an in-house breeding program. At 5 days post fertilization (dpf), larvae were selected and divided into 1-L tanks with a density of 100 larvae L^{-1} and cultured in static conditions. At 15 dpf larvae were transferred to a recirculation system in 3.5 L tanks and larval density adjusted at 35 larve·L^1 in order to maintain biomass density and promote growth. The experimental design included different feeding protocols in co-feeding with microdiet (md) and live feeds (*Artemia* sp.,Art nauplii; rotifers - *Brachionus plicatilis, rots*), a microdiet-based and a rotifer-based feeding protocol were tested for early (8 dpf) and extended transition (15 dpf) to a microdiet-based diet and at different feeding frequencies (3- or 4-times day^{-1}). In addition to influence of the duration of the co-feeding, we also evaluated the effect of the concentration of rotifers, for that we used two distinct concentrations, 50 rot/ml and 150 rot/ml. The feeding protocol started with an increment of 1mg md/day^{-1} until 15dpf, followed by an increment of 2 mg/day^{-1} until 30dpf. The growth performance was evaluated at 15 and 30 dpf.

Results

At 15 dpf it was possible to observe that larvae fed with the rotifer-based protocol showed the highest total length while larvae fed with a co-feeding with microdiet and *Artemia* nauplii, or microdiet with rotifers at 50 rot/ml or 150 rot/ml and an early transition of were the smallest (Fig. 1A). At 30 dpf larvae fed only with microdiet in a frequency of 3 or 4 times were smaller than all the other treatments, and larvae on co-feeding with microdiet and *Artemia* nauplii with an extended transition , or larvae on co-feeding with microdiet and rotifers with an extended transition and the larvae fed with a rotifer-based protocol were larger than all the other treatments (Fig.1B).

The survival at the end of the trial (30 dpf) was significantly lower in larvae on co-feeding with artemia and microdiet and early transition and in all the treatments where larvae were only fed with the microdiet (3, 4 or 5 times per day).

Discussion

The use of live prey is known to contribute to fish welfare since it triggers the natural predatory behaviour and consequently reduce stress in captivity (4,5). Based on our results it was also possible to observe that they are essential to promote better growth and survival.

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Treatment name	Composition	Brief description
Art_5_8	Art (5 art/ml) + md	2 meals of Art (until 8 dpf) + 1meal of md
Art_5_15	Art (5 art/ml) + md	2 meals of Art (until 15 dpf) + 1 meal of md
Rot_50_8	Rots (50 rot/ml) + md	2 meals of rots (until 8 dpf)+ 1 meal of md
Rot_50_15	Rots (50 rot/ml) + md	2 meals of rots (until 15 dpf)+ 1 meal of md
Rot_150_8	Rots (150 rot/ml) + md	2 meals of rots (until 8 dpf)+ 1 meal of md
Rot_150_15	Rots (150 rot/ml) + md	2 meals of rots (until 15 dpf)+ 1 meal of md
Rot only	Rots (150 rot/ml)	3 meals of rots
Md x3	Md	3 meals of md
Md x4	Md	4 meals of md

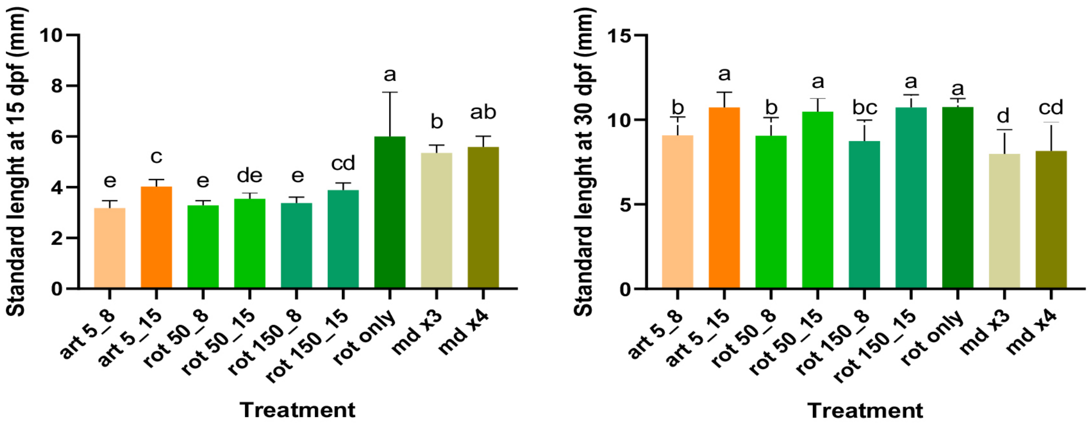

Figure 1: Effect of the different dietary protocols in zebrafish larvae length (A) at 15 dpf and at (B) 30 dpf. Data are expressed as mean ± SD. Statistical differences (One-way ANOVA followed by post hoc Tukey's test, $p < 0.05$) are represented by different letters.

Overall, our results revealed that a rotifer-based diet and extended co-feeding regime with *Artemia* sp. or rotifers until 15 dpf decreased the size dispersion among individuals and promoted larvae growth when compared to a microdiet-based feeding regime or an early transition to microdiets. Larvae survival also increased both in rotifer-based, early and extended co-feeding regimes, when compared to microdiet-based protocols where larvae are fed at least 4 times day^{-1}. In addition, the extended feeding protocol with microdiets showed to decrease the costs and labor associated with larval feeding and reduce the time spent in zebrafish maintenance when compared to microdiet-based protocols. In this work, it was possible to verify that zebrafish constitutes an important biological model for testing protocols for future applications in fish production. This work contributes to a better understanding of the impact of feeding regimes on larval growth and development and promotes the implementation of standardization procedures between different zebrafish facilities, towards higher replicability of scientific results.

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MINERALOGENIC AND IMMUNOGENIC EFFECT OF *Phaeodactylum tricornutum* EXTRACTS PREPARED FROM HIGH PRESSURE DISRUPTED BIOMASS

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Introduction

To adapt to diverse and extreme environmental conditions, marine organisms produce bioactive compounds with high potential for applications in fish farming and human health (Jha and Zi-Rong, 2004). From this large marine diversity, microalgae have received much attention recently because of their unique biochemical composition (Barbeiro, 2015) and innovative biological activities (Brillatz et al., 2018; Lauritano et al., 2020), but also because they can be easily cultivated in bioreactors. However, their potential is still largely underexplored. The diatom Phaeodactylum tricornutum is rich in eicosapentaenoic acid, monounsaturated fatty acids such as palmitoleic acid, minerals and different carotenoids such as fucoxanthin (Neumann et al., 2018) and is therefore a specied of choice for compounds with antioxidant, anti-cancer, anti-inflammatory, anti-obesity and anti-diabetic effects (Bae et al., 2020). In this work, we aimed to investigate the mineralogenic and immunogenic potential of P. tricornutum ethanolic and aqueous extracts using fish-derived in vitro cell systems.

Materials and methods

Biomass of *P. tricornutum* was produced by Allmicroalgae (Pataias, Portugal) and disrupted through exposure to high pressure at LNEG (Lisbon, Portugal). Disrupted biomass was macerated for 3 h in absolute ethanol or Milli-Q water at 100 mg/ml at four different pressures (A- cell disruption with 77.8%, B- cell disruption with 79.2%, C with 51% and D with 0%). To test the mineralogenic activity of the extracts, VSa13 cells were exposed for 21 days and mineral deposition was assessed through alizarin red staining quantification. Extract immunogenic activity was evaluated by qPCR for assessing gene expression of pro-inflammatory cytokines (*tnfα*, *il1b* and *il8*) and two enzymes with a role in the immune system (*sod* and *cox*) in SHK-1 cells exposed to extracts for 4 h.

Results

Samples A_E, B_E and D_E prepared in ethanol showed a statistically significant increase in extracellular matrix mineralization of 45%, 40% and 25%, respectively, compared to vehicle (Figure 1A). Among the samples prepared in water, only D_H exhibited a significant decrease in mineral deposition (approximately 10%), when compared to vehicles (Figure 1B). As expected, cells that were not exposed to the mineralogenic cocktail only showed residual mineralization of the extracellular matrix.

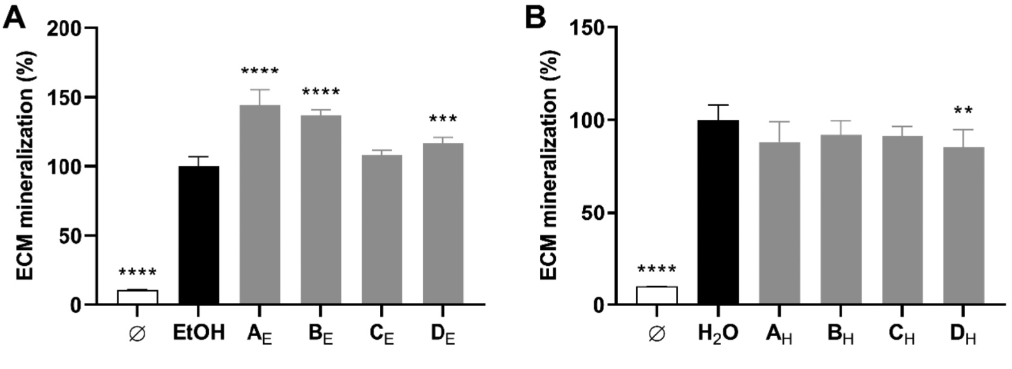

Figure 1. Effect of ethanolic (A) and aqueous (B) extracts of *P. tricornutum* on extracellular matrix mineralization. Ø, cells that were not exposed to the mineralogenic cocktail; EtOH, cells exposed to the mineralogenic cocktail and ethanol (negative control); H_2O, cells exposed to the mineralogenic cocktail and water (negative control); Statistical analysis using one-way ANOVA (**p<0.01; ***p<0.005, ****p<0.001). *n*=6.

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Exposure to extracts of *P. tricornutum* revealed that the relative expression levels of the *sod* gene was similar to the control for all the samples, while the relative expression of the other genes of interest (*il1b*, *il8*, *cox* and *tnfα*) had a slight increase, possibly indicating an increase in the immune system.

Discussion

The ethanolic extracts prepared from *P. tricornutum* led to a significant increase in the deposition of calcium phosphate crystals in the extracellular matrix of VSa13 cells, in particular extracts A and B, which were subjected to greater cellular disruption. The correlation between the mineralogical effect and the cellular disruption may indicate that bioactive compounds are intracellular and thus become accessible after an efficient disruption of the cell wall. In 2018, Grossmann et al. demonstrated that cellular disruption of *P. tricornutum* using high pressure allows obtaining extracts with molecules of interest. In general, the aqueous extracts did not show any effect on the deposition of calcium phosphate crystals despite sample D showing a small decrease in mineralization. The different mineralogenic effects of the extracts dissolved in water and ethanol can be explained by the fact that the solvents have different polarities, and thus, allow a selective extraction by solvent affinity, that is, depending on the type of solvent, the dissolved compounds will be different. Thus, the promineralogenic compounds are soluble in ethanol and not in the water, something already observed in extracts prepared from other marine sources. Ethyl acetate extracts from green marine macroalgae *Cladophora rupestris* and *Codium fragile* and from the marine plant *Spartina alterniflora* had a promineralogical effect on VSa13 cells, and this action was not observed in their aqueous extracts (Roberto et al., 2018; Surget et al., 2017). Regarding the immunogenic activity was possibly to observe an increase in the relative expression of the pro-inflammatory cytokines, important initiators of the inflammatory response (Kim et al., 2013). The fact that *sod* gene presented a different behaviour may indicates an antioxidant activity that prevents the toxic effects of oxygen radicals in cells, already observed by Wang et al(2018).

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OPERATION OF RAS UNDER HETEROTROPHIC-N ASSIMILATION: CARBOHYDRATE ADDITION TO FRESHWATER RAS WITH RAINBOW TROUT

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Introduction

All RAS rely on the common biological treatment process – nitrification – to avoid accumulation of ammonium. The process takes place in biofilter and has numerous assets and drawbacks. An alternative to autotrophic biofilters is heterotrophic N assimilation (HET-N) (De Schryver and Verstraete, 2009; Ebeling et al., 2006). In this process, heterotrophic bacteria consume ammonia directly for growth and thereby remove dissolved N. HET-N assimilation require a higher carbon to nitrogen ration (C:N ratio) which can be achieved by adding a bioavailable carbon source – i.e. acetate (OA) to the system. This can be done by adding easily bio-degradable carbon in the water to promote bacterial growth in the water. However, the addition of carbon and corresponding growth of heterotrophic bacteria leads to a large increase in oxygen consumption, increase in turbidity and high suspended solids loads (Crab et al., 2012). An alternative carbon source is slower releasing products like polyhydroxybutyrate (PHB) (Luo et al., 2020). These PHB pellets placed in a reactor may reduce the turbidity and suspended solids compared to direct carbon addition. The objective of this study was to compare traditional RAS with nitrifying biofilter with different modes of carbon addition to obtain heterotrophic N assimilation. The main focus included assessment of changes in water quality and implication on fish performance during a 4-week experimental period.

Materials and methods

Four different treatment groups were tested in triplicate in 1.7 m^3 pilot scale RAS:
1) RAS with biofilter (*Control*); 2) RAS with biofilter + acetate addition (*BF+OA*); 3) RAS without biofilter + addition of acetate (*OA only*) and 4) RAS without biofilter + PHB biopellets (*PHB only*).

Water samples were collected weekly during the duration of the trial and tested for different physical, chemical and biological parameters. Fish survival was compared and performance was assesses by weight gain during the trial.

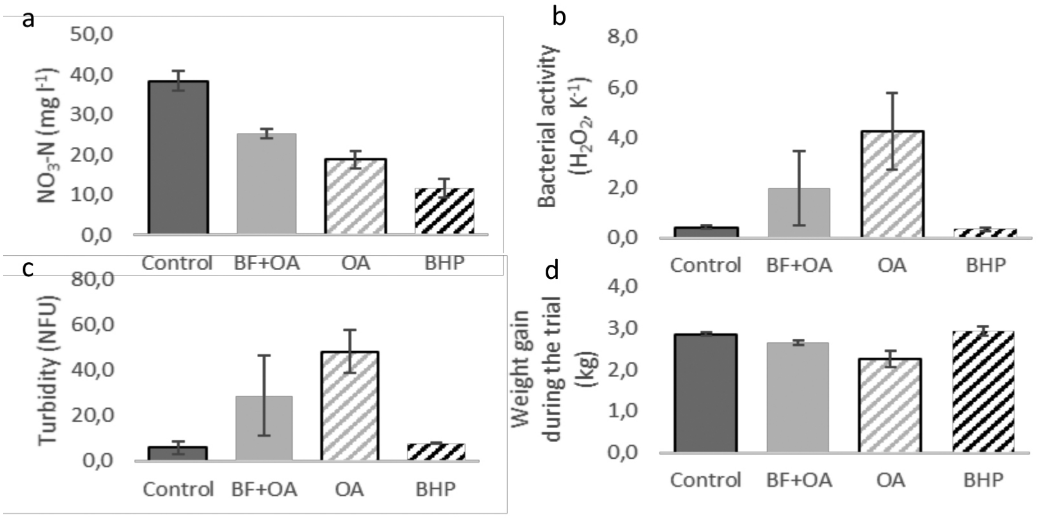

Figure 1. Different water quality and fish performance parameters at the end of the 4 week trial. (average values ± standard deviation). a) Nitrate levels b) Bacterial activity measured using H2O2 degradation method c) Turbidity d) Fish weight gain during the trial.

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Results and discussion

Control systems led to accumulation of NO_3 as predicted during the duration of the trial (Fig 1a). In contrast, all other RAS with carbon addition were found to have much lower levels of nitrate at the end of the trial, with 70% less NO_3 in the *PBH only* RAS. Despite not having any biofilters installed, both *OA only* and *PHB only* RAS maintained acceptable water quality parameters during their respective start up phases. The *OA only* RAS showed a moderate accumulation of NH_4+ during the first 2 weeks (up to 0.747 mg l^{-1} TAN), and a constantly higher level of NO_2 throughout the trial (0.834 mg Nl^{-1} vs approx.. 0.100 mg N l^{-1} in the control group). The *PHB only* RAS did not cause accumulation of TAN and only a moderate increase in NO_2- in the first week. This indicates a very fast start up time for Het-N systems, which could have important monetary implication for fish farms as it reduces start up time and allows for an increase feeding from earlier in production. The addition of acetate to the water (*BF+OA* and *OA only)* caused expected formation of bioflocs in the systems, and a significant increase in bacterial activity and turbidity (fig. 1b and 1c). While the fish in all tanks showed apparent appetite and readiness to eat, tanks where acetate was added (*OA only*) led to substantial feed spill. The feed spill may have been caused by the deteriorating water quality conditions affecting fish appetite, or could simply be due to visual impairment of the fish, reducing their ability to ingest the feed. In contrast, system fitted with biopellets (*PHB only*) remained stable and showed no changes in both physical (turbidity) and biological (bacterial activity) parameters during the trial. The absence of bacterial accumulation and no increase in turbidity in the water in *PHB only* suggests that the processes primarily occurred within the reactor, having no negative impacts on water quality.

These results translated to differences in the growth of the fish during the trial, with no differences found between the control groups and the biopellet group (fig. 1d), but a reduction in weight gained in both acetate groups (7,4% less growth in the *BF+OA* and 21 % less growth in the *OA* group). Survival was over 99%.

The results of this trial indicate that it's possible to convert a conventional RAS to Het-N assimilation through the use of different carbon sources, without compromising fish survival and reducing dissolved N and P in the water. However, the use of fast degrading carbon such as acetate resulted in deteriorating water quality and affected fish performance by significant reduced growth. In contrast, slow degrading carbon such as PHB biopellets in a reactor, resulted in reduced levels of N and P, with none to negligible effects on water quality and equal fish performance, while having a much faster start up time compared to traditional autotrophic biofilters.

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ANTIOXIDANT STATUS AND DISEASE RESISTANCE OF NILE TILAPIA (*Oreochromis niloticus*) FED DIFFERENT SOURCES AND LEVELS OF SELENIUM

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Introduction

The percentage of fishmeal and fish oil used in aquaculture diets has been decreasing over the past decades. However, with the increased production volumes of the aquaculture sector, the total usage of these marine-based components hasn't decreased. Since both fishmeal and fish oil are scarce raw materials, which are considered to be expensive and less sustainable, the need to implement alternative ingredients, mostly terrestrial plant-based materials, remains critical to ensure the further growth of the aquafeed industry. One of the issues when replacing marine-based materials with terrestrial plant-based materials is the decline of important micronutrients in the diet. One of these nutrients is selenium (Se). Se is an essential trace element, involved in several biological systems in the animal body. It plays a vital role in the protection against oxidative stress but also in growth, antioxidant status and immune response of animals. Se deficiencies can therefore lead to serious problems in aquaculture, whilst previous research has shown that the increased inclusion of plant-based ingredients has led to decreasing Se contents in both feed and animal tissues. Nile tilapia farming is increasing and becoming more and more intensive, with increased stress and disease pressure as a consequence. Supplementation of Se has become crucial to maintaining fish health and productivity. Additionally, decreasing amounts of Se in fish fillets due to the replacement of marine-based ingredients by plant-based ingredients reduces the healthy image of fish and shrimp for human consumption. To date, Se can be offered to fish in various forms, inorganic, such as sodium selenite, and organic Se, such as L-selenomethionine. Although it is known that these forms differ in bioactivity, bioavailability and efficiency the effect on fish health has not been researched intensively. The objective of this study was, therefore, to assess the effects of sources and levels of Se on the antioxidant status, immune response, and disease resistance of Nile tilapia.

Material and methods

A completely randomized design involving a 2 x 3 factorial arrangement treatment was used. Seven different diets were designed, where a basal Tilapia diet was used as a control diet. Six different treatments were obtained by supplementing the basal diets with 1, 3 and 5 mg/kg Se from L-selenomethionine (SeMet; Excential Selenium 4000 by Orffa Additives BV, The Netherlands) or with 1, 3 and 5 mg/kg Se from sodium selenite (SS). After fish feed production, total Se content in the diets was analysed (Table 1). A total of 735 Nile tilapia (13.52 ± 0.5 g) were randomly assigned to one of the 21 (seven treatments, in triplicate) tanks with 1,000 L of water, with 35 fish per tank. Fish were fed by hand to apparent satiation twice a day for 8 weeks. Moreover, blood samples were collected and analysed on blood chemistry including albumin, globulin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and cholesterol. Antioxidant enzyme activity such as catalase (CAT), superoxide dismutase (SOD), lysozyme (LZM), myeloperoxidase (MPO), glutathione peroxidase (GPx) lipid peroxidation and malondialdehyde (MDA) were also measured. Additionally, survival and growth performance during the feeding trial were measured. After the feeding trial, fish were injected intraperitoneally with *Streptococcus agalactiae*, and the 14-day cumulative mortality was calculated and compared amongst treatments.

Results and discussion

The blood serum biochemical data of tilapia fed the different treatments showed that ALT, AST and cholesterol were affected by both Se levels and source. Increasing dietary Se levels significantly decreased ALT and cholesterol ($p < 0.05$), whilst it significantly decreased the AST levels ($p < 0.05$). Next to that, it was observed that fish fed SeMet had significantly higher values of ALT were significantly higher compared to those fed SS ($p < 0.05$), whilst AST and cholesterol levels were significantly lower in fish fed SeMet compared to SS ($p < 0.05$). All three parameters are the first indicative indicators of increased resilience against oxidative stress and decreased lipid accumulation.

Fish fed diets supplemented with SeMet of 1.0 mg Se/kg resulted in higher growth performance as well as an improved antioxidant status of the fish (Figure 1). Dietary sources and levels of Se had a significant effect on lysozyme (LZM), catalase (SOD), myeloperoxidase (MPO), superoxide dismutase (SOD), and glutathione peroxidase activity (GPx) ($p < 0.05$; Figure 1).

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Table 1: Analysed Selenium contents of the experimental diets.

Diet	Se addition (mg/kg)	Total Se in diet (mg/kg)
Control	0	0.68
SeMet - 1	1	1.78
SeMet - 3	3	3.53
SeMet - 5	5	4.9
SS - 1	1	1.75
SS - 3	3	3.49
SS - 5	5	5.3

SeMet = L-selenomethionine, SS = sodium selenite

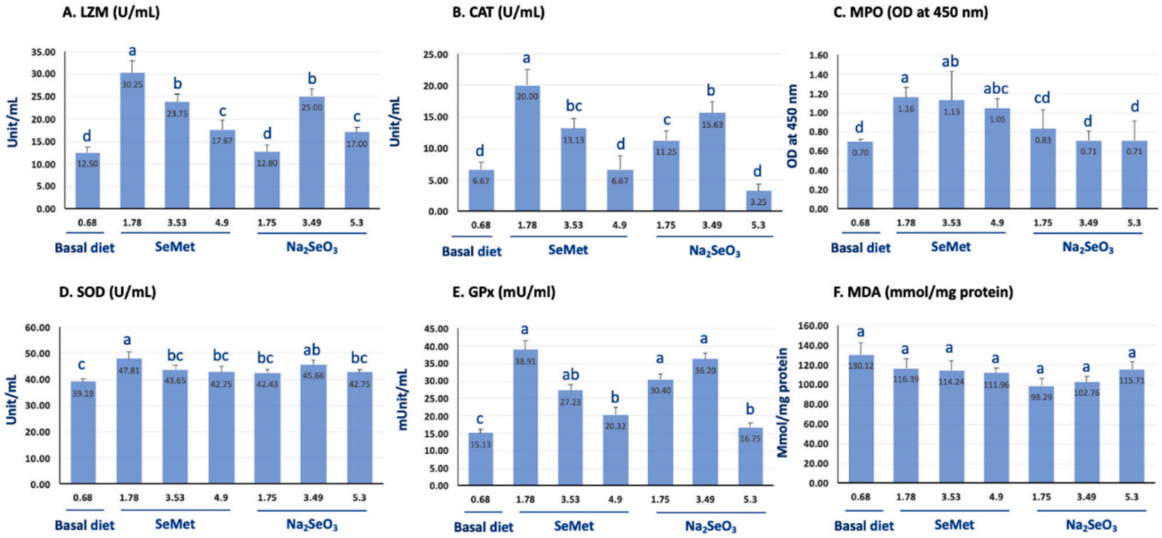
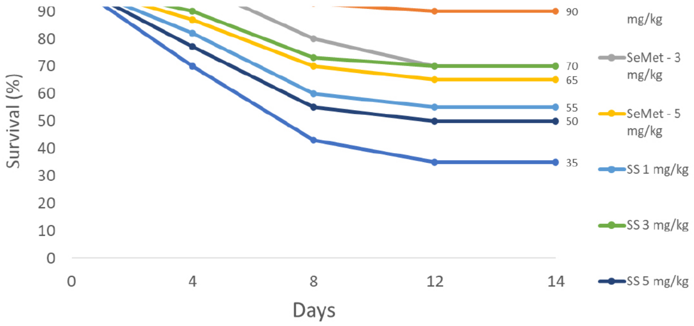


Figure 1: (A) Lysozyme activity (LZM), (B) catalase activity (CAT), (C) myeloperoxidase activity (MPO), (D) superoxide dismutase (SOD), (E) glutathione peroxidase activity (GPx), and (F) malondialdehyde (MDA) of Nile tilapia given different dietary sources and levels of mg Se/kg for 8 weeks.

Figure 2: Survival (%) of Nile tilapia fed different sources and levels of selenium after a *Streptococcus agalactiae* challenge.



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The potential benefits of dietary sources and levels of Se on the disease resistance of fish against *S. agalactiae* were also assessed (Figure 2). These results may indicate that Se supplementation enhances the immune response, which may be related to the increased resistance of Nile tilapia to pathogenic bacteria. Additionally it could be observed that supplementation of SeMet at an inclusion level of 1 mg Se/kg resulted in the highest disease resistance. The current research results show that dietary sources and levels of Se not only improved the growth performance, but also enhanced the immune response of Nile tilapia, indicating the significant importance of Se in improving growth and immunological parameters. Moreover, Se could be used in feed additives to enhance disease resistance and increase the host immune response against *S. agalactiae* infection. Not every source of Se gives the same performance. In this experiment, it was observed that the inclusion of organic Se (SeMet) outperformed the inorganic Se (SS) regarding antioxidant status, growth performance and immune response, with an optimum supplementation of 1 mg Se/kg from SeMet.

Conclusion

In summary, Se is an essential micronutrient for improving overall fish health and growth performance by improving the antioxidant status and immune response of Nile tilapia. The supplementation level of 1.0 mg Se/kg of organic Se (L-selenomethionine) is suggested to be the optimal level to improve growth performance, antioxidant status and immune response of Nile tilapia. Therefore, the results of the present study suggest that supplementation with L-selenomethionine has high potential in the aquafeed industry.

EVALUATION EFFECTS OF HEAT WAVES ON FARMED *Crassostrea gigas* BIOMETRIC INDICES, HEAT SHOCK PROTEIN 70, 72, 90 AND P53 GENE EXPRESSION

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Introduction

The whole shellfish farming sector is negatively affected by the heat waves (HWs). According to the predictive models developed by Galli et al., 2017, in the years 2041-2050 HWs will not exceed 28° C in the north Adriatic Sea, but there will be an increase in the duration of the wave up to 30 days. Knowledge regarding the effects of HWs on bivalves at physiological and molecular level is still limited. In order to fill this gap, this study tries to simulate what will happen in the future, assessing morphometric indices, *HSP70*, *72*, *90* and *p53* gene expressions in pacific oyster exposed to prolonged HW (Farcy et al., 2009)

Materials and Methods

120 specimens of farmed *C. gigas* reared into 12 aquaria were divided in two groups: the control one at temperature of 20±0,5°C and the heat wave exposed group at 28°±0,5°C. At 3, 5, 10, 30 days exposure, oysters were sampled in both groups to determine the biometric indices of whole animal weight, soft body and shell weight, the condition index (CI). At the last time sampling also hepatosomatic index (HSI) and the physiological index of clearance rate (CR) were evaluated. For each time point, the hemolymph was analyzed to determinate total protein, aspartate transaminase, ammonia, magnesium, calcium, phosphorus, and glucose concentrations. *HSP70*, *72*, *90*, (in muscle and gills) *p53* (only in gills) gene expressions levels were evaluated by real-time PCR in the heat wave exposed group as relative up- or down-regulation compared to the expression detected in the control group.

Results and Discussion

About biometric indices, significant differences were found for wet soft body weight and CI, where the lowest values were reported by the exposed group at 30 days. Also the HSI of the exposed group at the end of trial is lower than the control group, while there was no difference between groups in term of CR. Although the heat wave reduced meat yield and energy reserve of the stressed group, this filtered at the same rate than the control group. The HW affected some hemolymph biochemistry parameters like the calcium: there was a significant increase of the values in the exposed group over time, different also from the control group. The HW alters the ultrastructure of the valves, made of calcium carbonate, therefore the animals have sequestered more calcium from the environment and transported it through the hemolymph to the cells used for the shell formation (Knights et al., 2020) (Fig.1). In the muscle, *HSP70* and *HSP72* followed the same trend with the highest values at 5 days in the exposed group: these two *HSPs* induce the use of energy in response to stress (the muscle exposed to HW consumed more energy). *HSP90* in muscle and all three *HSPs* and *p53* in gills were upregulated in the exposed group at 10 days. Gills are the first organ that comes into contact with environmental stressors and, even if concave oyster has developed over time a functional adaptation to sudden environmental changes, prolonged exposition to HW caused an over-expression in *HSPs* and *p53* genes with an alteration in cellular metabolism of the tissue (Fig. 2).

Conclusion

Heat wave causes a reduction in the quality of the oyster as a food product due to the loss in pulp yield, energy reserve. Moreover, HW induces an over-expression of the genes involved in physiological stress/repair response and apoptosis altering cellular metabolism and animal welfare. In order to help farmers in mitigating the consequences of this acute climate events, it will be necessary to develop predictive systems able to warn farmers about the arrival of heat waves.

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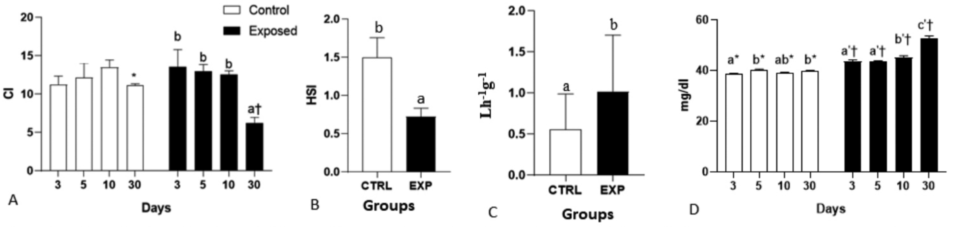

Fig. 1: Condition index (A) and hemolymphatic calcium (D) of the two experimental groups at 3, 5, 10, 30 days exposure; hepatosomatic index (B) and clearance rate (C) of the groups at 30 days.

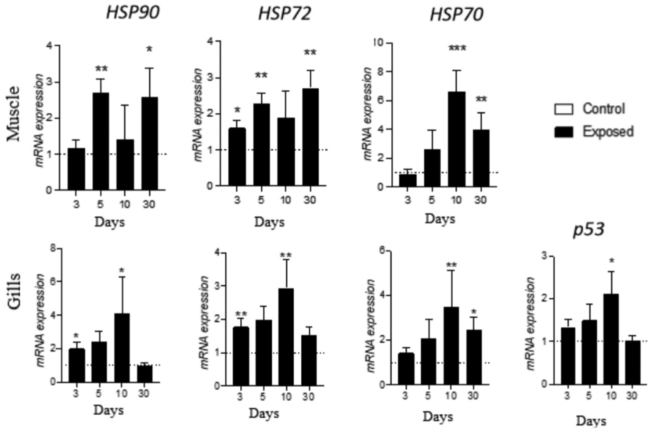

Fig.2: Temperature effect on *HSP70*, *72*, *90*, and *p53* mRNA expressions of the exposed group normalized on controls for each time point in muscle and gills tissues.

Acknowledgements

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INFLUENCE OF DIFFERENT COMMERCIAL DIETS FROM THE KINGDOM OF SAUDI ARABIA IN GILTHEAD SEABREAM (*Sparus aurata*) PRODUCTION: THE EXPANSION OF WELL SUCCEEDDED SPECIES TO THE RED SEA CONDITIONS

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Introduction

The gilthead seabream *Sparus aurata* is one of the most produced species in Mediterranean Sea and has a great potential of expansion for other regions were the species occurs. The species have been cultivated in the Red Sea in commercial farms, and studies on nutrition in the early grow-out phase and at harvestable size are always on demand. The Kingdom of Saudi Arabia has a large coastline suitable for aquaculture with favorable conditions and environment. Aquaculture is a growing sector in KSA and nutrition is one of the key factors, since feeds are responsible for up to 60% of the total production costs. So, in order to have the optimal feed conversion ratio and high feed efficiency, in the present work we evaluate the best diets commercially available in KSA and investigated the growth performance, survival and fish incorporation of the feeds in order to understand seabream performance in different sizes under the Red Sea conditions.

Materials and methods

In the first feeding experiment at the Coastal Marine Resources Labs (CMOR) of the King Abdullah University of Science and Technology (KAUST), 20 juvenile of gilthead seabream (Run 1) with an average body weight of 119.6 ± 8.9g were randomly stocked in 600 L tanks. In the second experiment, 30 pre-adult seabream (Run 2) with an average body weight 310.3 ± 41.9g were randomly stocked in 1m^3 tanks. For Run 1, the fish were fed with four commercial diets (diet 1, 2, 3, and 4) and for Run 2, three commercial diets were tested (diet 5, 6, and 7). Fish were hand-fed until apparent satiation two times per day, sampling was conducted every 2 weeks to determine growth performance and survival, and proximate composition of the diets was performed. The parameters that fulfilled the prerequisites of analysis of variance (ANOVA) were performed with a one-way ANOVA followed by Fishers Least Significant Difference (LSD) and the parameters that did not fulfill the prerequisites were compared using Kruskal-Wallis-H test followed by Student Newman Keuls using Sigmaplot version 11.0. The level of significance adopted were P<0.05.

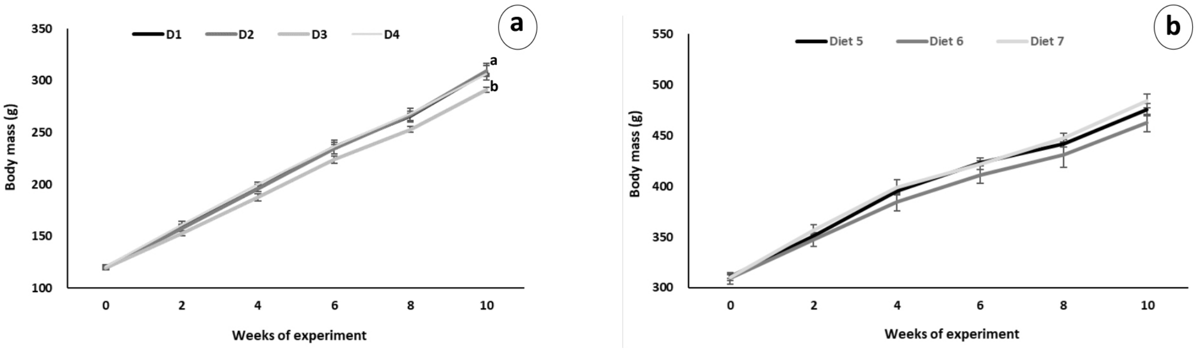

Figure 1. Average body mass of Sea bream *Sparus aurata* during the experimental period. a. Juvenile seabream fed with four commercial diets during 10 weeks. b. Pre-adult seabream fed with three commercial diets during a period of 10 weeks.

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Results

The survival of the gilthead seabream in both runs was around 98.3-100% and showed no significant difference among the different diets tested. In this study, the 119 g juvenile gilthead seabream showed a maximum daily weight gain of 2.36 g day^{-1} and an SGR of 1.27% day^{-1}. Comparing the different diets, the FCR value was significantly lower (more efficient) in diets 2 and 4 as compared to diets 1 and 3, so, the higher energy diets, showed better FCR. For the pre-adult gilthead seabream (run 2), the FCR value was significantly lower ($p<0.05$) in diet 6-fed fish (1.95) as compared to fish fed the diet 5 (2.16) and 7 (2.02). Diet 3 exhibited lower body mass at the end of the experiment ($p=0.001$) (figure 1a), while diet 4 showed the highest PER. In the pre-adult fish, diet 7 showed the highest SGR and growth gain, while, diet 6 showed the lowest FCR, but no differences was observed in the body mass (figure 1b).

In the juvenile fish, the feed intake was highest in diet 1 that has the lowest energy content while the lowest feed intake in diet 4, which has the highest energy.

Discussion

These growth results are comparable or even better to the results obtained by previous researches wherein juvenile gilthead seabream of about 105-254 g size cultured at salinity conditions lower than the Red Sea (<41 ppt) attained a daily weight gain of around 1.15-2.87 g day^{-1} and an SGR of around 0.56-0.96% (Ribeiro et al., 2015). The FCR values obtained from this study (1.57-1.7= juvenile; 1.95-2.16= preadult), were also comparable from previous reports (1.5-2.1) (Araújo-Luna et al., 2018; Ribeiro et al., 2015).

It has been observed even with other fish species that the energy density of the feed directly influences the amount of feed consumed, such that a high energy diet can result in a reduction in the amount of food eaten by the fish. In many fish species, that protein (nitrogen) retention can be improved by partly replacing dietary proteins with lipids resulting in a protein-sparing effect (Santinha et al., 1999). PER, which is a good measure of the protein-sparing effect by non-protein energy sources, showed a significantly higher PER in diet 4, suggesting that the lipid in the diet has probably spared protein. Our result for diet 4 is comparable to previous results wherein PER was highest in a lower protein (40%) as compared to a higher protein diet (55%) but with a higher energy diet (Santinha et al., 1999). However, optimum lipid content should be determined in the diet considering that a high-energy diet generally leads to fat deposition and thus can affect the fish commercial value.

Conclusions

The growth results from this study showed comparable with past researches despite the fish were grown in slightly higher temperatures (>24°C), probably due to the higher protein and/or energy in some of the diets tested. Our results suggest that gilthead seabream can attain high survival in the Red Sea with good growth performance and FCR levels showing potential economic feasibility for a commercial operation.

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INFLUENCE OF DIFFERENT COMMERCIAL DIETS FROM THE KINGDOM OF SAUDI ARABIA IN SOBAITY SEABREAM (*Sparidentex hasta*) AQUACULTURE: INVESTIGATING A POTENTIAL CANDIDATE SPECIES FOR WARM WATER AQUACULTURE

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Introduction

The Sobaity seabream *Sparidentex hasta* also known as silver porgy is considered one of the promising fish species for aquaculture, especially in the Arabian Gulf region, because of its good adaptation in captivity, rapid growth, high market value, tender flesh and rich flavor. When compared to other sparid species, sobaity seabream is not so well studied and not informations regardins growth performance and feeding conversion ratio (FCR) nor nutritional requirements are available in order to advance in the production of the species. The Kingdom of Saudi Arabia (KSA) has a great potential for aquaculture production due to the good conditions of the Red Sea and Arabic Gulf. Therefore, knowing that nutrition is one of the key factors in fish production and represents major expenses of the total cost of production, our main goal was to study Sobaity seabream growth performance, FCR and survival fed with four diets commercially available in KSA in order to investigate Sobaity performance under the Red Sea conditions.

Materials and Methods

For the first feeding trial (Run 1) at the Coastal Marine Resources Labs (CMOR) of the King Abdullah University of Science and Technology (KAUST), 60 juvenile of Sobaity seabream (Run 1) with an average body mass of 66.0 ± 1.4 g were randomly stocked in 600 L tanks. In the second experiment, 34 pre-adult seabream (Run 2) with an average body mass of 331.0 ± 3.9g were randomly stock in $1m^3$ tanks. For Run 1, the fish were fed with two commercial diets (diet 1 and 2) and for Run 2, three commercial diets were tested (diet 3, 4, and 5). Fish were hand-fed until apparent satiation two times per day and sampling was conducted every 2 weeks. We evaluated the growth performance and survival. Data were tested for normality and equality and one-way ANOVA and Kruskal-Wallis-H test were tested according to the parametric and non-parametric data using Sigmaplot version 11.0. The level of significance adopted were P<0.05.

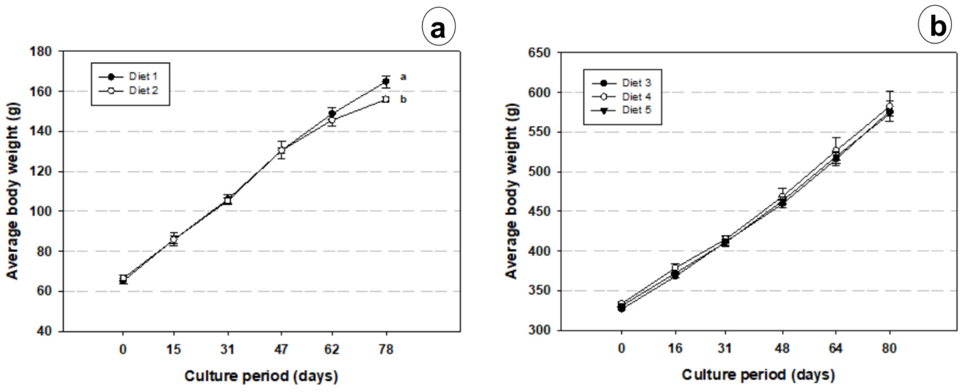

Fig. 1. Average body mass of Sobaity seabream *Sparidentex hasta* during the experimental period. **a**. Juvenile seabream fed with four commercial diets during 10 weeks. **b**. Pre-adult seabream fed with three commercial diets during a period of 10 weeks.

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Results

The survival of the Sobaity seabream in both runs was around 96-99% and showed no significant difference among groups. In the juvenile fish, the final body weight (run 1) was significantly higher ($p<0.05$) in diet 1 as compared to diet 2 (figure 1a). The growth increased from 65.3 g to 164.9 g, with a total weight gain of 99.7 g, with a daily weight gain of 1.3 g day^{-1} and a specific growth rate (SGR) of 1.2% day^{-1}. In the pre-adult (run 2), the final body weight, total weight gain, daily weight gain and SGR showed no significant difference between the three diets tested (figure 1b). The juveniles total feed intake was significantly higher in diet 1 when compared to diet 2 (134.8 g fish^{-1}), but for the pre-adult, no significant difference was observed. The FCR in the juvenile, no difference was observed and the value was around 1.5, while in the pre-adult, diet 2 group was significantly lower than the other diets and values were around 1.6-1.4.

Discussion

The present study showed that sobaity seabream is a very good candidate species for increasing aquaculture production in the Red Sea and Arabian Gulf region, since the species exhibited good growth performance that is close to past-developed research; however, improvement on the nutritional characteristics of the feed is essential to improve the growth performances of the fish. In the present study, the daily weight gain was 1.3g day^{-1} and SGR of 1.2% day^{-1} in a 66g fish, which is better than previous research where a 51g juvenile growth 0.85 g day^{-1} and 0.76% day^{-1} of SGR (Houssain et al., 2014). The species attained good growth performance and low FCR when compared to previous study where FCR obtained was 1.6-2.57 (Houssain et al., 2014). For the pre-adult, commercial diet 2 showed significantly better FCR and PER, which shows the high protein requirement of the species. However, the percentage of protein of diet 2 was 46% which is lower than the recommended for the species in previous work that mention diet with 50% protein (Mozanzadeh et al., 2017), so studies approaching lower and sustainable protein sources and alternative to fish meal can be explored in order to produce a cost-effective diet for the species.

Conclusions

Sobaity seabream is a highly recommended species to increase aquaculture production in Middle East region, especially in the Kingdom of Saudi Arabia. The species exhibited a very good growth performance, survival and if the nutritional requirements of the species is targeted in future research; sobaity can be a great potential candidate species to increase marine fish production in KSA.

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THE DEVELOPMENT OF MACHINE VISION TO MEASURE SEA URCHINS

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Introduction

Sea urchin aquaculture (echinoculture) is an emerging industry with high potential. To facilitate production optimisation, accurate and effective methods for quantifying somatic growth of urchins are required. The method frequently utilized involves manually measuring diameter of each individual urchin test (shell) with vernier callipers. This is not only time consuming but also has potentially high measurement variance. Recently, a 3D laser scanning technique was developed (Shpigel and Erez, 2020), which provides highly accurate measurements but requires urchins to be sacrificed and is time consuming. Both methods can be applied when quantifying small sample sizes. However, these methods are not practical when measuring large quantities of urchins. As echinoculture develops to a commercial scale, methods will be required to monitor urchin size accurately and effectively. This study compared the use of callipers to a newly developed machine vision technique for measuring test diameter of two urchin species. Machine vision has been highly effective at optimising fish aquaculture (Saberioon et al., 2017), but does not seem to have been applied significantly in other aquaculture sectors.

Methods

Urchin test diameter (TD) was manually measured using vernier callipers to the nearest 0.5mm. Fifty *Tripneustes gratilla* were measured manually by three operators, with operator 1 conducting 3 measurements of the urchins and operator 2 and 3 conducting one measurement each. This allowed for measurement variance to be determined within a single operator and between operators. Twenty *Parechinus angulosus* were measured separately three times by a single operator. Each urchin was numbered to ensure the same individuals were compared. Images of each numbered urchin were captured and inputted into the machine vision model, with 10 urchins per image. Three images were taken of each set of urchins, but the position of numbered urchins was changed randomly between each image.

To ensure accessibility of this machine vision program, no specialised equipment was used. Images were acquired using a simple homemade photo box and mobile phone cameras. Specific instructions on the setup can be found in the Read.me file (Batik and de Vos, 2022). To check for instrument bias, repeated images of urchins were taken with two different mobile phones, a Samsung A52 and Huawei P30. The program was written in Python3 primarily using the OpenCV library. The machine vision model measured urchin TD by computing the contour of each urchin, using the Canny contour detection algorithm, and the corresponding minimum area bounding rectangle. An HSV filter and basic morphological operations (i.e. blur, erode, dilate) were applied to remove the spines from the measurement area. Settings for the latter used in this analysis are set as defaults; however, these can

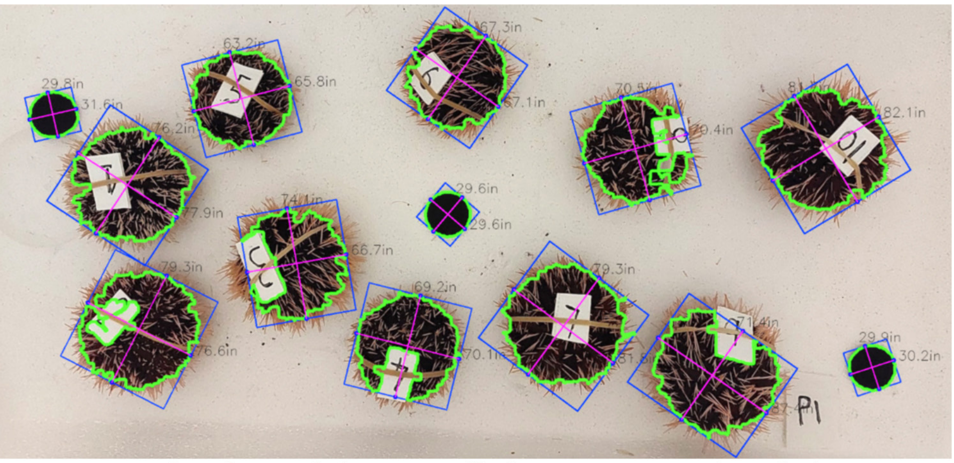

Figure 1. Results of one of the *T. gratilla* images once run through the program. Numbering system on urchins caused some issues with edge detection, measurements affected by this were excluded.

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Statistical analysis was conducted in R. To compare measurement methods, the coefficient of variation (CV) between each measurement was determined within each measurement technique. Each technique was regarded a treatment and an ANOVA and Tukey tests were applied. To compare the two different phone cameras' effects on measurements, a paired T-test was used. All assumptions were checked and met.

Results

For *P. angulosus,* the CV for machine vision measurements was 2.21% and lower than that of the manual measurements by a single operator with a CV of 2.65%, however there was no significant difference ($F_{(1,39)}$=0.791, p = 0.379). For *T. gratilla* the machine vision (Fig. 1) CV was 1.55% and significantly lower ($p < 0.001$) than that of the calliper measurements taken by multiple operators (CV of 2.41%), but not significantly different to that of a single operator (CV of 2.02%, p=0.068). There was no significant difference in measurements between phones (p=0.655).

Discussion

The machine vision program was able to provide more accurate measurements of urchin diameter than manual calliper measurements, and with much higher efficiency. It proved difficult to configure the image in such a way that only the urchin test (shell) was measured and not the spines as well. The higher accuracy of the program with *T. gratilla* than *P. angulous* was the result of colour variation between *P. angulous* individuals (colours include black, orange, red purple and white), making it difficult to fit into generalist parameters of the colour filter. While it was still relatively accurate, and now the default parameter should work for urchins of most colours, there is still much scope for refinement and optimisation of the script for specific species. There was decreased accuracy due to mobile phones not having a truly flat lens. This could be improved by using more appropriate cameras, although this would reduce accessibility. While there were no significant differences between mobile phones, it is still recommended to use the same phone and setting across all data points. A possible extension could be real time measurements to attach to a grading machine. In conclusion, this program could be a useful and accessible tool for anyone measuring the diameter of live urchins.

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FLAT OYSTER AND SEAWEED AQUACULTURE AND OFFSHORE OYSTER RESTORATION IN THE BELGIAN PILOT OF THE H2020 UNITED PROJECT

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Introduction

Growing demand using European seas for renewable energy, food production, tourism and transportation has resulted in increasing competition for marine space with the push to move into offshore waters. The Belgian pilot within the European Union's Horizon 2020 project UNITED[1] (Multi-**U**se offshore platforms demo**N**strators for boost**I**ng cost-effec**T**ive and **E**co-friendly pro**D**uction in sustainable marine activities) seeks to combine the cultivation of seaweed (*Saccharina latissima*) and European flat oyster (*Ostrea edulis*), and the restoration of flat oyster reefs within an offshore windfarm operated by Parkwind. To do so, best cultivation and restoration practices to withstand the harsh environmental conditions were identified in the nearshore test site at Westdiep, a NATURA 2000 area five kilometres off the coast of Nieuwpoort. Only the best suited materials and methods will be applied in the offshore site in the Belwind offshore wind farm, 46 km off the coast of Zeebrugge.

Materials and methods

The installation of the four offshore oyster restoration structures was conducted early summer 2021. Four restoration structures filled with nature-inclusive scour materials were placed on the scour protection of two monopiles. At each monopile, two structures were installed. At the first monopile, adult European flat oysters (*Ostrea edulis*) were added. At the monopile downstream of the first one (with respect to the main current direction), no oysters were added. The goal of this is to verify whether settlement on the scour protection materials and reef restoration can be enhanced by adding adult oysters.

For the flat oyster cultivation, several techniques were tested, such as oysters cemented or glued to ropes and sticks, and OSTRIGA lantern baskets connected to a rope.

To identify best cultivation practices for *Saccharina latissima* (known as sugar kelp) in the highly exposed environment of the Belgian part of the North Sea (BPNS), different substrates, seeding techniques (comparing direct seeding versus the importance of a nursery period), and strains were investigated. Two types of seaweed cultivation nets (AtSeaNova) were partly pre-seeded with two different strains of *S. latissima* gametophytes and kept in a nursery setting for four weeks. The remaining part of the nets was seeded with the direct seeding method a day before being installed at the nearshore site (Westdiep) in November 2020. Biomass, density, maximum length and width of *S. latissima* were assessed upon the harvest in May 2021.

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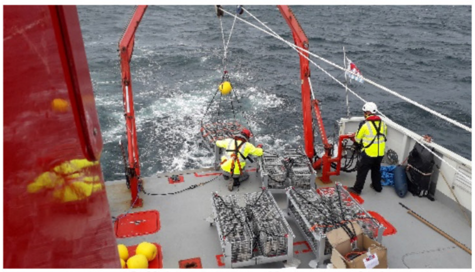

Figure 1 : Offshore installation of the oyster restoration structures filled with nature-inclusive scour protection, shell materials and adult flat oysters to evaluate success of flat oyster settlement and reef formation on the different materials.

*Figure 2 : Healthy looking sugar kelp (*Saccharina latissima*) during the spring inspection in April 2022 at the nearshore site (Westdiep, 5km off the coast of Nieuwpoort)*

Results

The offshore restoration of *O. edulis* reefs gave very promising results. Successful settlement of juvenile flat oysters was discovered on both the scour protection and the added shell materials.

Concerning oyster cultivation nearshore, the oysters cemented to ropes initially seemed to be a practical cultivation method. However, the ropes were overgrown with fouling organisms, mostly mussels once the mussel spat season had begun and water temperatures had risen. The OSTRIGA baskets on the other hand proved to be successful, although pinpointing of the amount of stacks, maze width, and depth was necessary. The other systems seemed to be unpractical (too heavy, basket loss, too much fouling) and need further tweaking before commercial upscaling and/or implementation in a wind farm can be considered.

The first seaweed cultivation trials resulted in a promising harvest of *S. latissima* in May 2021 (Figure 2). While seaweed biomass was of good quality with barely any visible epiphytes, strong biofouling especially on substrates seeded via direct seeding was observed, resulting in low biomass (0.1 kg m^{-1}). Best results were achieved with nets incubated in the nursery before deployment (1.1 kg m^{-1}).

Discussion and conclusion

Overall, the implementation of offshore multi-use activities is still in its infancy. Test pilots such as the Belgian pilot are a prerequisite before offshore multi-use can take place. While nearshore testing of systems and techniques are needed before moving offshore, the real test will come with the installation of offshore seaweed and oyster aquaculture inside an offshore wind farm. The first results of the offshore oyster reef structures in the Belgian pilot show at least that this environment is suitable for the survival, growth, reproduction and settlement of European flat oysters, which looks promising for successfully implementing oyster restoration inside offshore wind farms in the BPNS. The first seaweed cultivation trials made clear that proven cultivation protocols from sheltered locations need adaptation for the successful implementation in the highly exposed offshore environment. Future planned activities on the installation of the aquaculture structures offshore will prove possible further success.

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THE PROBIOTIC STRAIN *Shewanella putrefaciens* Pdp11 STRONGLY MODULATES GENE EXPRESSION OF THE FISH PATHOGEN *Vibrio harveyi*

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Introduction

Shewanella putrefaciens Pdp11 was isolated from the skin of healthy gilthead seabream (*Sparus aurata*) and has been studied as a probiotic in the culture of some fish species, such as *S. aurata* and *Solea senegalensis*. These are two relevant species cultured in the Mediterranean area, but the pathogens that affect them represent a serious problem for the commercial development of their farming industry (Cámara-Ruiz *et al*., 2020). *Vibrio harveyi* is a significant pathogen of cultured marine fish, especially in southern Europe, and causes a wide variety of pathologies in its hosts (Zhang, He and Austin, 2020). Studies on SpPdp11 probiotic showed antagonistic activity against *V. harveyi in vivo* (Cámara-Ruiz *et al*., 2020), so there is an interest in understanding the mechanisms underlying the observed effects in order to enhance them. In this work, the interaction between SpPdp11 and *V. harveyi* was studied by RNA-seq to understand how SpPdp11 interferes with the pathogen through bioinformatics analysis.

Material and methods

Three types of cultures were performed for this study: SpPdp11 alone (code: Pdp11), *V. harveyi* alone (code: Vibrio) and SpPdp11 and *V. harveyi* together (code: PV). Aquafeed medium was used for all the cultures, which is composed of 160 g/L feed + M3 minimal saline medium. Liquid cultures of SpPdp11 and *V. harveyi* were brought to a known concentration of equal numbers of CFU/mL and 100 µL of the source flasks were added to solid plates of aquafeed medium. For the interaction, a 1:1000 concentration of *V. harveyi*:SpPdp11 was assumed based on previous studies of their growth curves. All cultures were incubated for 24h at 23ºC and samples were stored in TRIsure™ until processing. RNA extraction was performed with the GeneJET purification kit (Thermo Scientific™) following the manufacturer's instructions.

Sequencing libraries (paired-end, 2x75 bp) were constructed at the Ultrasequencing Service of the University of Malaga and sequencing was performed on the Illumina NextSeq™ 550 platform. Six samples of each experimental group (Pdp11, Vibrio and PV) were sequenced. Raw reads were processed using a bioinformatic pipeline including pre-processing with SeqTrimBB (v2.1.8), mapping with BWA (v0.7.5), differential gene expression with DEgenesHunter (v1.0) and functional enrichment of GO terms with in-house R scripts. GO enrichment was performed for the three ontologies: Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). For the processing of Vibrio data, in addition, a de novo assembly of the genome and its functional annotation were performed, and predicted genes were searched for in the core genome of Vibrionales order (results not shown). Data processing was carried out with the computational resources of the Andalusian Bioinformatics Platform. To find out the effects of the interaction in both species, two gene expression comparisons were carried out: PV vs Pdp11 and PV vs Vibrio. Genes were considered as differentially expressed (DEG) if fold change > 2 and adjusted *p*-value < 0.05. A network analysis was performed for the most relevant functional enrichment results using Gephi (v0.9).

Results

When comparing PV vs Pdp11 through differential expression analysis, 66 DEGs were obtained, of which 31 were upregulated and 35 were downregulated. Significant GO terms (*p*-value < 0.05) were obtained in BP and MF ontologies, but not in CC. Nevertheless, due to the low number of DEGs obtained, few genes were annotated in the enriched terms and no further analysis was performed.

When comparing PV vs Vibrio, 2370 DEGs were obtained, of which 1113 were upregulated and 1257 were downregulated. Significant GO terms were obtained in the three ontologies for both upregulated and downregulated genes, but the enrichment yielded higher significance values in the case of the upregulated genes than of downregulated genes. The most relevant results were obtained in BP ontology, so a network analysis was performed in order to study the distribution of up and downregulated genes and enriched terms. Briefly, upregulation of expression was observed in genes related to motility, localization, organization and peptide metabolism, while downregulation was observed in genes related to stress response, signal transduction, transcription, transport and metallic clusters assembly.

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Discussion

A quick glance at the results shows that the number of deregulated genes as a result of the interaction is much higher in *V. harveyi* than in SpPdp11. This suggests that the presence of SpPdp11 affects *V. harveyi* to a greater extent than *V. harveyi* affects SpPdp11. Considering that *V. harveyi* is a pathogenic strain and SpPdp11 is a probiotic strain, this may be positive for its probiotic capacity, as it not only maintains its functionality almost intact, but also produces a huge imbalance in that of *V. harveyi*. Although the pathogenicity mechanisms of *V. harveyi* remain to be properly resolved, many virulence factors have been identified, as hemolysins, proteases, lipopolysaccharide (LPS), the capacity to bind iron, interaction with bacteriophages, biofilm formation and quorum sensing (Zhang, He and Austin, 2020). Interestingly, we found that genes related to metallic clusters assembly were downregulated in *V. harveyi* in the presence of SpPdp11, which could be modulating this virulence factor. Moreover, this has been found to be important for pathogenicity in fish, but not in invertebrates (Owens, Austin and Austin, 1996). As for other groups of downregulated genes, such as those related to stress response or transcription, it would be necessary to elucidate how they are related to known virulence factors. Overexpressed genes are mostly related to two major functionalities, protein biosynthesis and motility. SpPdp11 is clearly affecting motility and organization of *V. harveyi*, but the consequences of this deregulation need to be elucidated. Regarding protein biosynthesis and translation, it is well described that *V. harveyi* uses many proteins as virulence factors (Zhang, He and Austin, 2020), so an experimental study on the expression of known virulence factors under conditions of interaction with SpPdp11 would be desirable. Based on these results, new interaction experiments will be conducted to observe the behaviour of the two species at the interaction front and metabolomics and proteomics studies to explore further effects of the deregulation of gene expression.

Acknowledgments

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FROM LONG-ESTABLISHED CONSUMER TO RESPONSIBLE PRODUCER: MONITORING BENTHIC AND SEDIMENT IMPACTS OF A SHELLFISH OFFSHORE AQUACULTURE PROJECT IN ONE OF EUROPE'S FAVORITE MUSSEL MARKETS

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In a context of increasing demand for food and decreasing resources availability, aquaculture emerges as a promising sector. With an annual growth rate of 5.8% since 2001 (FAO 2018), it is one of the fastest growing industry. Mussel aquaculture, in particular, is considered to be one of the least impactful food production method as it usually grows native shellfish species which take their nutrients directly from the environment, a process that makes them interesting bioremediation tools against water eutrophication. In addition, shellfish aquacultures have a potential for carbon absorption and can provide shelters for fish and marine life. Mussels also constitute a healthy source of food for consumption, further raising the attention that is being drown to it. However, while the incentives for shellfish aquacultures are increasing, the potential impacts of such farms, especially the more extensive offshore ones, should not be overlooked.

With an average consumption of 2.69 kg/inhabitant, Belgium is the third favorite market for mussels in Europe (FAO 2014). The country purchases 30 000 T of mussels each year at a high cost (83 560 000 €). Long-established purchaser, Belgium is the only main consuming country in Europe that is not also a main producer (FAO 2014). Although a few other attempts have failed in the previous decades, a new innovative longline offshore mussel aquaculture is being installed off the coast of Nieuwpoort. The first harvest of mussels is expected for 2023, and in a second and third phase, the aquaculture will also produce oysters and seaweeds, mainly aimed at the Belgian internal market.

In this poster, we present a monitoring plan in order to assess the environmental impacts of this aquaculture in the Belgian part of the North Sea for the next 9 years. While this study is integrated in a wider pluri-disciplinary research, we here focus on the monitoring of the potential impacts on the benthos as well as on the seabed and sediment dynamics and geochemistry.

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THE EFFECTS OF STOCKING DENSITIES VERSUS TANK VOLUME ON THE SKELETON OF GILTHEAD SEABREAM *Sparus aurata* IN THE HATCHERY AND PREONGROWING PHASES OF THE PRODUCTION CYCLE

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Introduction

Gilthead sea bream (*Sparus aurata*) production is one of the main aquaculture industries in the Mediterranean, producing 258,754 tones of seafood in 2019 (FAO, 2021). However, in recent years uncertainty regarding the profitability and the economic losses have been inevitable for many production facilities due to rapid market expansion in the 1980s followed by an oversupply establishing a lower market value in the last two decades (Llorente et al., 2020). Therefore, a focus on increasing the production value rather than increasing production quantity would be a sustainable solution to improve profitability and adjust for long-term environmental and economic goals in the EU (Llorente et al., 2020).

The coupled application of Large Volumes (Ø =30-60m^3) and low densities (< 16 larvae/L) has been demonstrated (Koumoundourous et al., 2004; Boglione et al., 2009; Prestinicola et al., 2013) augment the survival rate and the morphological quality of gilthead sea bream and other Sparids. However, the separate effects of density or volume, decoupled from each other has not been investigated. This knowledge will help farmers to produce subadults of higher quality to be ongrown by modulating only one of these two factors, without any need for extra economic investment, high-tech solutions, or new tanks.

The aim of this study was to individuate which between ‘large volume’ and ‘low density’ is the main driver in attaining high quality gilthead sea bream during both the hatchery (from eggs to juveniles) and the preongrowing ($W_{average}$ up to ~55 g). The experimental design envisaged to test the effects at a commercial scale of A) larger and smaller tank volumes on seabream, stocked at the same density; and B) higher and lower stocking densities on seabream maintained in the same tank volume. The experimented tank volumes were smaller, and the densities higher than those tested in previous studies. The choice of the experimental tank volumes (500 vs 1000 L) was based on the ubiquity of these tanks in almost every Mediterranean farm. The densities we utilized were those indicated as interesting to be tested by API (Italian Association of Fish farmers).

Materials & Methods

Experimental rearing were conducted in the EcoAqua facilities at the University of Las Palmas, Gran Canaria (Spain) for the hatchery phase and at the Intituto Portugues do Mar e Atmosfera facilities in Olhão Portugal for the preongrowing phase. 3 different densities (Low Density (LD): 25 eggs/L and 5kg/m^3; Medium Density (MD): 125 eggs/L and 10kg/m^3; High Density (HD): 250 eggs/L and 20kg/m^3) were utilized for the hatchery and ongrowing phases respectively. Two tank volumes were tested for each density condition, in all the trials: 500L tanks (small volume) and 1000L tanks (large volume). Natural seawater was pumped into the systems and all of the rearing parameters were maintained the same for all of the conditions, save the volume or the density. Additionally, oxygen was maintained at above 70% SAT for both trials. Seabream were reared for approximately 2 months in each trial.

Juveniles from the hatchery phase were whole-mount stained with Alizarin red while the sub-adults from the preongrowing cycle were radiographed. Monitoring of skeletal anomalies was done for both studies using an adapted alphanumeric code to account for skeletal elements affected and region of body in which the anomaly was located Prestinicola et al. (2013). Data was expressed in a raw matrix in order to calculate the frequencies of anomaly types found over the total amount of anomalies and a binary matrix to calculate the frequencies of individuals affected by every anomaly types. All statistical analyses and graphs were done using Python and Past 4.02 (Hammer et al., 2001).

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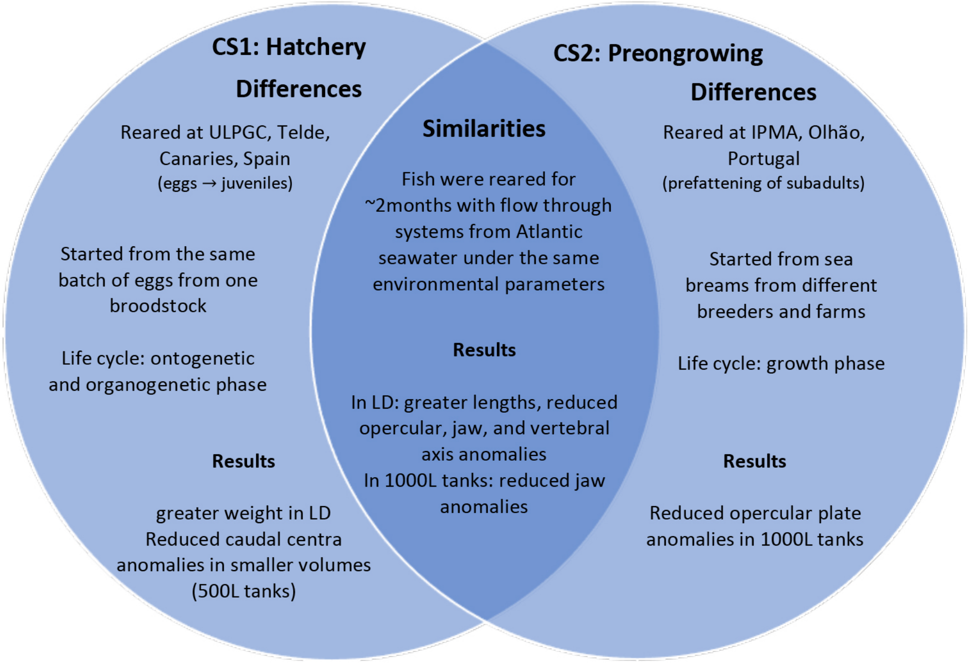

Figure 1. Venn-diagram displaying differences and similarities between the two case-studies.

Results

Strikingly the environmental parameters of varying degrees in density and volume elicited similar responses in both early juveniles and subadults.

Both experimental cycles enhanced significant greater lengths, reduced opercular, jaw, and vertebral axis anomalies in LD reared seabream, while larger volumes reduced the incidence of jaw anomalies. This outcome highlights the predominant effect of environmental drivers on skeletal plasticity in this species, regardless of notable differences in genetic origin, life-stage, and ontogenetic phases (Fig. 1).

The possible hypotheses (behavioral, chemo-physical, physiological, etc…) that can be formulated to explain this primary, more positive effect of low stocking density rather than the larger tank volume, are largely discussed.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 766347, BioMedAqu, ETN 766347.

FINE MAPPING FOR VIRAL NERVOUS NECROSIS RESISTANCE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) IDENTIFIED BY WHOLE GENOME SEQUENCING

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Introduction

Viral Nervous Necrosis (VNN) is the most impacting disease for the European sea bass industry, which can lead to mortality up to 90%. This virus develops favourably in warm waters and outbreaks may be promoted due to global change-related increase in temperature in coastal and marine environment. Identifying concrete improvements in European sea bass populations resistance to VNN is thus of utmost importance in the light of a potential upsurge in viral outbreaks. Selective breeding is a promising strategy to reduce the frequency and severity of VNN outbreaks. Several genomic regions (Quantitative Trait Loci or QTLs) have been identified thanks to SNP arrays, as involved in the variation of VNN resistance, named (Palaiokostas et al., 2018, Griot et al., 2021, Faggion et al., 2022). With the development of cost-efficient sequencing tools, the identification of QTLs can be now much more precise. This work presents a next level of the genome-wide association studies (GWAS) using whole genome sequencing and imputation in order to refine the description of the genetic architecture of VNN resistance in European seabass.

Materials and methods

A total of 5,799 European sea bass (*Dicentrarchus labrax*) from two French breeding companies, EMGi and FMDS, were used in our study. The sires and/or dams used in the crosses of the commercial populations were sequenced, on a NovaSeq sequencer (Illumina), in order to know all the genetic variants characterizing both populations. Then, to study survival to VNN, all offspring were experimentally challenged to VNN on the SYSAAF-ANSES Fortior Genetics platform (ANSES, Plouzané, France), to evaluate their resistance, and each dead or alive individual were genotyped on the ThermoFisher 57K DlabCHIP SNP chip (Griot et al., 2021). We analysed sequences with a home-made bioinformatic analysis pipeline. First, the variant calling was processed according to the DeepVariant best practice and after classical filtering steps nearly 1 million SNPs shared by both commercial populations were identified. Second, FImpute v2.2 software was used to obtain an imputed genotype for these 1 million SNPs for each of the 5,799 offspring. Then, GWAS was performed for each population separately, using a Bayesian sparse linear mixed model (BSLMM) on a dataset of these imputed SNPs for all challenged individuals, using GEMMA software.

Results and discussion

A genomic region (QTL) of 250 base pairs on LG12, common to the two commercial populations, was detected with strong evidence for association with the phenotypic trait of VNN resistance. We identified a survival rate of nearly 78% for individuals possessing the resistance allele at this QTL, vs. 40.6% for the individuals with the susceptibility allele (Figure 1).

Investigations are on their way to identify the genes and possible causal polymorphisms of VNN resistance in this region. Identification of a causal gene would be a major step towards understanding immunological and physiological mechanisms of VNN resistance, and a major advance for breeding programs, allowing for greater genetic gain by using marker assisted selection. Further research is needed to validate the association of the polymorphism with resistance in other populations, and to confirm the mechanism implied using transcriptomics, proteomics or RNA interference.

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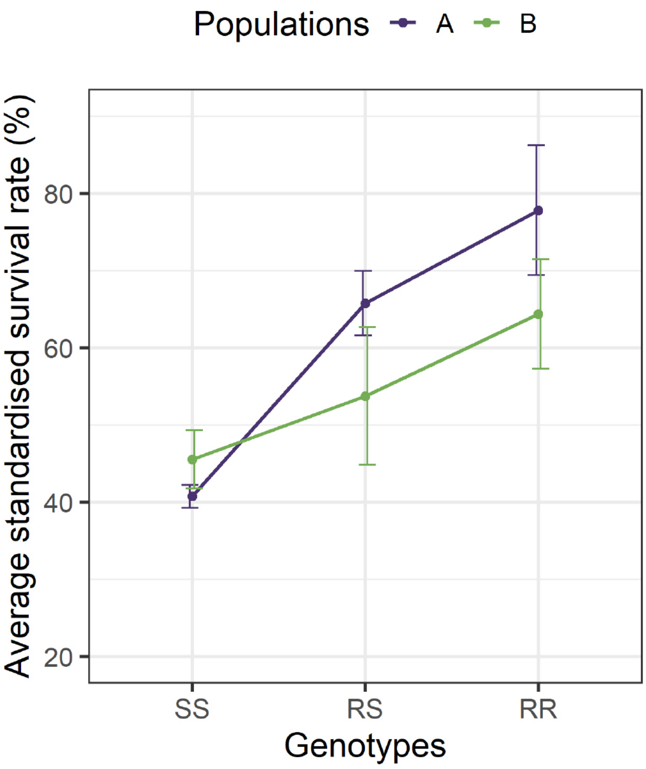

Figure 1: Average standardized survival rate for the strongest SNP detected with the BSLMM approach for the LG12 QTL. Each point is a mean of the standardized survival rate obtained in each of the commercial populations. The standard deviation was reported for each genotype in each population.

Acknowledgements

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THE ROLE OF UKRAINIAN AQUACULTURE IN RESTOCKING OF WILD *Acipenseridae spp.* POPULATIONS AND DEVELOPMENT OF COMMERCIAL STURGEON AQUACULTURE IN UKRAINE

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Ukraine is the one of countries of origin for a number of *Acipenseridae* species.

Some of them have been probably lost in the wild nature conditions. Such species as *Acipenser nudiventris* and *Acipenser sturio*, despite the fact that these species have been historically noted as registered for Ukrainian waters, don't occure in Ukrainian wild nature no less then three decades. At the same time wild populations of such species as *Acipenser gueldenstaedtii, Acipenser stellatus, Acipenser ruthenus, Huso huso* are stll presented in the Ukrainian marine and freshwater bodies.

Any sturgeon fishery is absolutely prohibited in Ukraine since 2000, and the all *Acipenseridae* species, historically described for the Ukrainian water bodies, have been included to the National Red Book in 2009.

An important step towards implementation of the strategy for conservation and restoration of sturgeon populations was the adoption of the «Action Plan for the Conservation of Sturgeons (Acipenseridae) in Ukraine for 2021-2030», approved by the Ministry of Environmental Protection and Natural Resources of Ukraine in 2020. This Action Plan provides implementation of number of important measures for conservation sturgeon wild populations.

Taking into account, that last decades only Danube is keeping a status of important sturgeon spawning freshawater body for anadromus sturgeon species, artificial sturgeon reproduction always had an extremely high importance for restocking sturgeon wild populations in the Azov Sea basin and in the Black Sea basin.

Historically, based on natural circumstances, Ukraine is conducting artificial reproduction of wild sturgeon populations mostly on the Black Sea basin. Here Ukraine has well functioning Dnieper sturgeon hatchery, which is producting and releasing up to 1.6 million sturgeon fingerlings yearly. Released sturgeon species are *Acipenser ruthenus* (the most in general fingerlings amount), *Acipenser gueldenstaedtii* and *Acipenser stellatus*.

Additionally to sturgeon restocking in Dnieper area, two releases of *Acipenser ruthenus* fingerlings to Danube have been done last decade: 2 thousand specimens in 2015, and 10 thousand specimens in 2021. These restocking actions have been technically realized by Ukrainian private aquculture company "Odessa sturgeonbreeding complex", and in 2021 it has occured in cooperation with the World Wide Fund for Nature Ukraine (WWF-Ukraine). About 1 thousand of released juveniles were marked with special external visual labels.

Also 2 thousand *Acipenser ruthenus* fingerlings were released in April, 2022, to Dniester river, as a result of fruitful collaboration of aquaculture company «ISHKHAN» with Chernivtsi National University, with support of Ukrainian governmental and non-governmental nature protection organisations.

Unfortunately, developing wild sturgeon populations restocking activities in Ukraine have met a strong obstacle as Russian agression against Ukraine, which has began since February, 24, 2022. Nowadays the Dnieper sturgeon hatchery is situated inside of the area of active military actions, and it excludes normal functioning of this hatchery current year.

Regading commercial sturgeon aquaculture, most developing aquaculture technologies using in Ukraine for commercial sturgeon breeding are freshwater cages farms (located at different river reservoirs) and high intensive recirculating aquaculture systems (RAS).

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Food products of sturgeon aquaculture have always been classified as a delicatessen with limited demand due to its high market value. During the USSR years commercial sturgeon farming in Ukraine was practiced in pond's, cage's and flowing pools' fish farms (about 15 sturgeon breeding farms in total). The main objects of cultivation in the ponds were Bester (*Huso huso X Acipenser ruthenus*) and paddlefish (*Polyodon spathula*), and in the cages and pools - Bester, sterlet (*Acipenser ruthenus*) and Siberian sturgeon (*Acipenser baerii*). The sturgeon production in Ukraine in 1985-1990 was ranged from 130 to 180 tons per year.

The 90s of the 20th century were a crisis for Ukrainian aquaculture and, above all, for its intensive forms. Due to a significant decline in the purchasing power of the population there was a reduction in demand for products of sturgeons. This led to the shutdown of a number of intensive aquaculture enterprises. By 2000 the number of sturgeon fish farms had fallen from 15 to 8.

With the beginning of the new century there is a gradual revival of sturgeon aquaculture. This was facilitated by the growth of demand for fish products and the emergence of full-fledged fish feeds of leading European brands in the domestic market. In 2011 there were about 10 sturgeon farms, including cage's and RAS. In 2021 were already more than 20 sturgeon farms, and at least half of this number were functioning as cage's and RAS aquaculture systems. Over the last decade, there has been a trend of increasing the number of farms that combine farming of table sturgeon products with the production of edible black caviar with use of modern methods of ultrasound diagnosis of sex and maturity of fish. Currently yearly sturgeon production in Ukraine is up to 100 tons, according to the official monitoring system.

The problems of sturgeon aquaculture development in Ukraine are, in particular:
a) There is still insufficient demand in the domestic market for an expensive table sturgeon products. Ways of solutions: the use of modern technologies and materials in sturgeon farming to reduce the cost and market price of fish and enter the markets of other countries.

b) Lack of production of quality fish feed in Ukraine. Feeds of domestic brands are still considered to be worth than imported feed at the expense of «price-quality», and starter feeds are not yet produced in Ukraine. The problem could be solved by the construction of a feed plant in Ukraine by one of the world's leading feed producers, such as BioMar, AllerAqua or Coppens. The expediency of the step is due to the fact that today in the intensive aquaculture of Ukraine for feeding fish use more than 2,000 tons of high-protein feed each year, and this amount is growing permanently.

c) The development of cages sturgeon (and not only) farms restrains the legally difficult procedure of renting part of the area of water reservours of national importance. Since the adoption of the Law on Aquaculture in 2012, only one farm has achieved to lease of part of the Kaniv Reservoir for cage sturgeon farming. However, currently the State Agency of Melioration and Fisheries of Ukraine does much efforts especially to solve this problem by implementing needed changes to the national legislation.

d) A significant number of aquaculture enterprises do not report on their activities, what causes lack in collected aquaculture data, especially in part of total production estimation. As the one of important keys to avoid this problem considered an improvement of existing responsibility rules, what is also the one of challenges for the State Agency of Melioration and Fisheries of Ukraine.

e) There are almost no subsidies from the state to support the development of aquaculture. However, due to investments in the aquaculture sector, a significant increase in production and quality of sturgeon products is possible.

f) Internal isolation of many aquaculture farms in their activities. Weak links with scientific institutions and limited access to world aquaculture practices do not contribute to the progressive development of production. One of the ways to solve this problem is to create non-governmental aquaculture joint ventures to solve common problems and gain access to modern technologies and other achievements of science and world practice.

As it should be concluded, the development of sturgeon aquaculture both restocking hatcheries and commercial sturgeon farms is an important factor of conservation and restocking wild *Acipenseridae spp*. population in natural water bodies of Ukraine and, at the same time, covering market demand on products of sturgeons. Huge water potential in Ukraine is an evident positive circumstance for sturgeon production growing. Besides, Ukrainian scientists and fish breeders have multiannual experience on sturgeon aquaculture for different nature and artificial conditions.

EFFECTS OF SPACE AVAILABILITY ON THE SKELETON AND SPATIAL DISTRIBUTION OF MEDAKA, *Oryzias latipes*

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Introduction

Differently from terrestrial farmed animals, there are currently no regulations on the minimum space which is necessary to fulfil the animals' needs in aquaculture. Crowding and reduced space under intensive rearing conditions are among the causative factors of skeletal anomalies, stress, and reduced animal welfare in farmed fish. In contrast, lower rearing densities improve survival, yield larger fish, and reduce the incidence of skeletal anomalies (Boglione et al., 2014; Izquierdo et al., 2010; Koumoundouros et al., 2001; Prestinicola et al. 2013; Roo et al., 2010). Behavioral studies on rainbow trout demonstrated that high stocking density has a significant effect on swimming activity, oxygen consumption and muscular activity, with substantial effects on swimming trajectories and space utilization (Bégout Anras 2004; Cooke et al. 2020). High rearing densities increase the incidence of particular skeletal anomalies in the model species medaka, *Oryzias latipes* (Di Biagio et al., 2022) and zebrafish (Martini et al., 2020). The laboratory setup provides a convenient and easily accessible monitoring of fish behavior that can provide basic information about the consequences of crowding for skeletal development and animal welfare. Thus, it was tested whether increasing the tank volume has an impact on reducing the incidence of skeletal anomalies and affecting medaka's behavior and survivorship. The present study represents a first attempt to combine anatomical observations and behavioral parameters, such as the overall space occupation (even utilization of the available space, independently of the spatial distribution) and the distribution (aggregated *versus* non-aggregated) of the individuals over time.

Materials and Methods

Medaka were reared from 0 to 40 days post hatching (dph) in 3L (small volume, SV) or 6L tanks (large volume, LV) at high density (40 medaka/L). All the experimental trials were carried out under standard rearing conditions (T: 26°C, photoperiod: 14H light: 12H dark, fed *ad libitum* with the commercial feed ZEBRAFEED® by Sparos, Portugal). The oxygen level was maintained between 98-100%. The levels of ammonia, water hardness and pH were controlled weekly. 150 min videos of the LV and SV tanks were taken every 4 days. At the end of the experimental rearing, the individuals were euthanized, fixed and whole mount stained with Alizarin Red S. Standard length S_L and skeletal anomalies were monitored. The video analysis was performed in triplicate on videos recorded from 40-, 38- and 35-dph of both tanks (LV and SV). The algorithm was written in MATLAB®.

Results

On a laboratory scale, the increase of available space (water volume) by maintaining high rearing density conditions seemed to not rescue or mitigate the effects of high density that were previously published (Di Biagio et al.). In particular, the high-volume condition significantly reduced survival, did not affect the length nor reduced the prevalence and distribution of skeletal anomalies. As an attempt to explain these results and correlate medaka's behavior with the changes in the available space (crowding), we investigated the overall space occupation and spatial distribution of the individuals over time. A first observation of the medaka juveniles swimming in tanks of different volumes reveals that the individuals do not exploit the entire available space. They rather tend to be grouped at the water surface with a subset of individuals that gathers at the bottom. The occupation of space and the dynamic behavior over time were investigated based on the image density of the recorded videos. The section of the imaged tanks was subdivided into three regions: top, central and bottom. Next, it was tested whether the density of the picture was homogeneous in these three regions by randomly sampling the same number of subregions, calculating the average of the pixel values (i.e. the image density) and testing whether the medians

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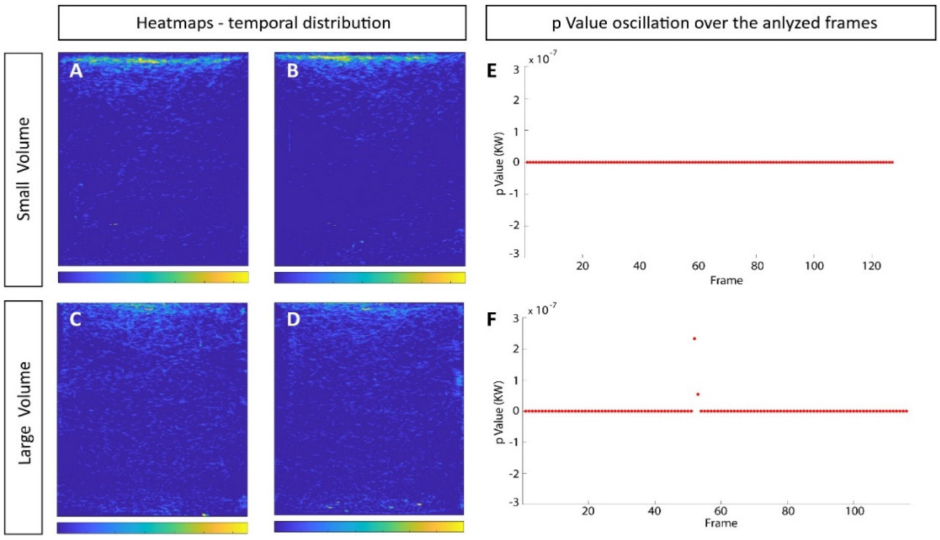

Fig.1 Representative heatmaps of the short-lived behavior of the individuals in small (A,B) and large (C,D) volume tanks. The blue-yellow range indicates the max and min pixel values calculated over 1 minute. (E-F) Scatterplots of the p-values over 90 min: 40 dph (LV) and 38 dph (SV). Each frame on the y-axis is representative of 1 minute of the analyzed video.

of the three regions were homogeneous. To evaluate the overall space occupation, all frame values were summed up in a single image irrespectively of the relative positions of the medaka juveniles. The Kruskal-Wallis (KW) showed significant differences between the medians. The calculated p-values for 40-, 38- and 35-dph indicate that the density of the top, center and bottom of the tank are not equal. Visually, the lack of uniform distribution is evidenced by the heatmaps (Fig.1 A-D). The second analysis focuses on multiple observations over time. Thus, the sequence of frames extracted from every minute of the investigated videos was examined to determine the homogeneity of pixel values distribution with the same regional approach. The KW test was applied to each group of frames (representative of 1 minute) and the oscillation of the calculated p-value was observed. None of the p-values exceeded the value of 0.05 for both rearing conditions (Fig.1 E, F). Thus, it can be assumed that the medakas are never randomly distributed in the tank, but rather grouped, confirming the visual observations.

Acknowledgements

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ENVIRONMENTAL PARAMETERS INFLUENCING VALVE MOVEMENTS OF MANILA CLAM FARMED IN VENICE LAGOON

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Introduction

Recently, mass mortalities of Manila clam, *Ruditapes philippinarum*, were verified in the lagoons of the North Adriatic Sea. In summer, they could be linked to thermal stress from heatwaves (Bertolini & Pastres, 2021). High mortalities in autumn may be due to the low salinity levels, caused by extreme precipitation events and related high freshwater discharges. This study aimed at elucidating the sublethal effects of temperature and salinity on Manila clam behaviour using the valvometer "SmartShell", a system developed by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (Di Giacinto et al, 2021).

Methodology

Fifty (50) *R. philippinarum* specimens were sampled in Venice Lagoon, Chioggia (VE). The first acclimatization period lasted 1 month in automated system (400 Liter aquaria) at 20 °C with artificial seawater (Instant Ocean®). Microalgae suspension of a mixture of *Tetraselmis* spp. and *Dunaliella* spp. were used for animal feeding. Experimental setting up was settled in four experimental groups composed by 2 individuals inserted in the SmartShell sensors in closed aquaria at different temperature and salinity conditions:1) 20°C ± 2 °C at 22 psu; 2) 20°C ± 2 °C at 36 psu; 3) 28°C ± 2 °C at 33 psu; 4) 20°C ± 2 °C at 33 psu (control group). The test lasted 14 days, during the first 7 days the behaviour of all molluscs was pre-evaluated at control conditions, then they were put under different salinity and temperature conditions. Four replicates were carried out.

Data were grouped in five classes of valve gapes (VG), namely: VG ≤ 20%, 21–40%, 41–60%, 61–80% and ≥ 81% (Redmond et al., 2017). Both in control and exposed mollusc groups, the average amount of time percentage spent in each VG class has been evaluated. Preliminary analysis showed some effects on periodicity, therefore Fourier analysis was used to analyse differences in periods and amplitudes of oscillations. Fourier analysis was done for each individual for both the pre-exposure and exposure periods separately.

Results and discussion

All molluscs had clearly distinguishable periods of activity and inactivity, repeated within the 24 hours. This was confirmed with the periodograms showing the highest peak around the 24h. In the low salinity group this value was slightly lower indicating a slightly higher frequency of oscillations. In the high temperature treatment, the molluscs spent more time opened than the control group, with the amplitude of oscillations significantly dampened.

In the control group, molluscs showed a flapping behaviour, spending more time at VG category of 61–80%. At lower salinity, the animals spent more time at VG of 61–80%. The most represented VG categories at higher salinity and temperature were 61–80% and 41 – 60%, respectively.

These results, first for clams, confirmed that high temperature influenced the behavioural patterns of molluscs, consistently with results for other species (e.g. mussels, Bertolini et al. 2022).

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FORESHELL PROJECT: DEVELOPMENT OF SANITARY/WEATHER ENVIRONMENTAL PREDICTIVE TECHNOLOGICAL TOOLS TO ENHANCE THE EFFICIENCY AND SUSTAINABILITY OF SHELLFISH FARMING

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Introduction

Mussel farming takes place directly in the marine environment. So, it is strongly influenced by weather and environmental conditions. In the last years, climate changes are causing severe weather events and anomalies of chemical/physical seawater parameters (e.g., higher average temperatures). Such anomalies are responsible of product losses, structural damages and, sometimes, the closure of production areas due to hygienic conditions. In fact, during the severe rain events, the rivers can discharge faecal bacteria into the sea, that can be accumulated by bivalves posing a potential risk to their consumers (Colaiuda et al, 2020). To face these emerging threats, FORESHELL project (funded by the FLAG Costa Blu trough 2014-20 EMFF program of the Abruzzo Region) created a management tool, able to forecast the main hydro-meteorological-environmental risk factors by alerting the farmer 48 hours in advance. The general aim was to enhance the efficiency and the sustainability of shellfish farming.

Methodology

The study area was a mussel farm located in the Giulianova Maritime District (Abruzzo, Italy). The hydrographic basins of the rivers close to the farm, Vibrata and Salinello, were constantly monitored trough the hydrological model (CHyM) (Tomassetti et al, 2005; Colaiuda et al, 2020; Lombardi et al, 2021), to forecast occurrences of discharge peaks at the river mouth. A specific sampling program was conducted before and after severe weather events to determine the concentration of *Escherichia coli* in freshwater at the river mouths and in mussels/seawater at the farming area. At the same time, the predictive tool was developed by acquiring hydrological data for sanitary risk and environmental parameters (such as sea water temperature, salinity, chlorophyll a, sea currents dissolved oxygen and wave height) though satellites and in situ probe. Furthermore, the growth of mussels was constantly monitored with biometric controls.

Results and discussion

A total of 10 hydro-meteorological events were investigated. At river mouth of Vibrata River the *E. coli* concentration was higher than legislative threshold (500 UFC/100 ml) (Italian Ministry of Health, Decree of 30 March 2010) following 7 hydro-meteorological events; whereas in Salinello River following 4 events. *E. coli* concentration was higher than the legislative limit (230 MPN/100 g) (EU Regulation n. 2019/627) in mussel samples following 4 events. The value of the bacterium in sea water ranged from 0 to 67 UFC/100 ml. Finally, the mussels showed a self-purification time ranged from 3 to 6 days. During the project lifetime the mussel grew constantly.

As regards Salinello River, the analysis showed that 100% of the samplings with high concentrations of *E. coli* in both molluscs and river water occurred following a significant increase in the river flow, while Vibrata River in the 93% of samplings. Based on these results, various flow rate thresholds were defined as an alert system associated with color codes and with the identification of different alert states: early warning (yellow), moderate alert (orange) and high alert (red) in order to make the whole system easily interpretable by the aquaculture.

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A customised oceanographic model, developed starting from satellite data, to estimate environmental parameters together with flow rate data of Vibrata and Salinello rivers estimated by CHym model were integrated into AquaX platform. The infrastructure of the AquaX platform was exploited to notify through alarms sent via SMS or email the forecast of adverse weather-marine events and anomalous fluctuations of the monitored physical-chemical parameter, with a predictive capacity of 48 hours. The alarms for the area investigated were set on the basis of bibliographic data and on the basis of aquaculture farmer's experience over the time

In conclusion, the FORESHELL project supplied farmer with easy-to-use tool useful to plan daily activities to obtain a sustainable and optimized production by implementing adaptive procedure to climate changes and ensuring health product.

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WILD LUMPFISH AS A MODEL TO INVESTIGATE NUTRITIONAL REQUIREMENTS OF FARMED LUMPFISH (*Cyclopterus lumpus)*

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Introduction

Sea lice infestations represent the major damaging parasite in salmonid aquaculture. Among the sea lice treatments, the use of cleaner fish, mainly lumpfish (*Cyclopterus lumpus*) and wrasse (*Labrus bergylta*), has increased exponentially in the past few years. Lumpfish is widely used as a cleaner fish by the salmon industry in Canada, north of Norway, Scotland, and Faroe Islands due to its higher cleaning efficiency in cold water compared to the wrasse. However, although the use of lumpfish represents a green alternative against sea lice, high mortalities are reported in the weeks post deployment and while they are in the cages. They are mainly caused by poor health and welfare, infectious diseases and the lack of optimal nutritional requirements and tailored diets (Brooker et al., 2018). This study aims to assess key differences between farmed and wild lumpfish regarding welfare and nutritional status and identify nutritional deficits in the lumpfish feeds in order to formulate a new feed that improves their survival.

Materials and Methods

The lumpfish for this study were collected at salmon farming sites, lumpfish hatcheries and from the wild. Regarding the wild specimens, both coastal and pelagic lumpfish were sampled for a total of 172 fish. Coastal lumpfish were collected along the coast, shaking seaweed, and using a hand net. Pelagic lumpfish were sampled by pelagic fisheries and by a research survey conducted in the Faroe Islands. 391 farmed lumpfish data and samples were collected at Atlantic salmon farming sites across the Faroe Islands, during regular lumpfish health monitoring. Twenty lumpfish in the sea cages were harvested with a hand dip net from the pen edges. Finally, thirty pre-deployment lumpfish were collected from two Faroese hatcheries. At each sampling occasion, a suite of morphometric measurements and operational welfare indicators (OWIs) were recorded for every fish, e.g., body condition, fin damage, deformities, eyes integrity, skin status, liver colour and stomach content, using the method reported by Eliasen et al. (2020). Both livers and whole fish were frozen and stored at -80°. Further nutritional analyses were carried out on livers, such as total lipid content, fatty acid methyl esters (FAME), lipid classes, total carotenoids, and on whole fish and feeds, such as proximate composition, fatty acid, and amino acid profile. A subsample of ten livers, anterior and distal intestine, spleen was fixed in 10% neutral buffered formalin for posterior histopathological analysis and in RNAlater for gene expression studies. Nutritional and histological data were analysed in R version 4.1.2. A principal component analysis (PCA) was carried out to identify the variables that gives the highest variation among groups and the visualization using R packages, FactoMineR and factoextra. Linear models with fish origin as independent variable and nutritional or histological parameters as dependent variables, were used to identify the key differences between farmed, hatchery and wild lumpfish.

Results

Preliminary data analysis focused mainly on lumpfish <50 g from different origins. Both lipid and protein content of whole fish was significantly different between fish origins, with the hatchery fish having a higher amount of lipid (2.9%) and protein (8.94%) compared to the farmed and the wild ones (1.11, 1.59%; 7.6, 7.99%). PCA was carried out on fatty acid profile of livers and whole fish. The first two components (PC1 and PC2) accounted for 80.4% of the variation and the fatty acids that give the highest contribution to the variability were 18:4n-3, 18:1n-9, n-3PUFA, 22:6n-3 (DHA), and 20:5n-3 (EPA). Significant differences were found in almost all the amino acids in wild fish compared to the hatchery ones. Preliminary results of liver histological analysis shows that liver vacuolisation is lower in wild (37%) than in farmed fish (43%). Livers from farmed fish also have higher liver inflammation, fibrosis, and congestion. Proximate analyses on different lumpfish commercial feeds show a substantial variability in both protein (45 to 56 %) and lipid (12 to 17%).

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Discussion

Lumpfish deployed in the sea cages displayed a higher variability on body composition compared to the ones in the hatcheries and the wild ones. Deployed lumpfish have access to both salmon and lumpfish feeds, different seasonal preys and the salmon cages are exposed to different environmental conditions (Imsland et al., 2015). Fish from the hatcheries are reared in controlled environmental conditions (Powell et al., 2018) and have a higher amount of lipid and protein content, and this reflects the diets they get in the hatchery. Wild lumpfish show overall a lower amount of lipid and protein in their body and a different fatty acid and amino acid profile due to their natural diet.

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TRANSIENT- AND RESIDENT-GUT MICROBIOTA IS POSITIVELY IMPROVED ON RAINBOW TROUT (*Oncorhynchus mykiss*) FED WITH INSECT MEAL FROM BLACK SOLDIER FLY (*Hermetia illucens*)

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Introduction

In aquatic animals, gut microbial communities shift with host development and living environments. Understanding the mechanism by which the environment impacts the gut microbial communities of aquatic animals is crucial for assessing and managing aquatic ecosystem health. Initially, fish embryos develop in a relatively constant bacteria-free environment (i.e., within the egg), although some environmental microbes quickly colonize the egg surface from the surrounding water after spawning. After hatching, environmental microbes colonize the gut and after the first feeding, new microbial communities are introduced into the gut with the diet, increasing microbial diversity. The gut microbiota further shifts with host development and with changes in diet. At an early stage, the gut microbiota is influenced mainly by the introduction of environmental microbes with water and diet; however, as the immune system and nutrition metabolism develop, gut microbes are selected and enriched gradually and can be altered with nutritional elements. For this reason, the research project aimed to explore the impact of novel ingredients on these gut communities using metabarcoding analysis of feed- and gut-associated microbial communities.

Materials and Methods

Feeding trial and sampling: the feeding trial was set at a recirculation aquaculture system (RAS) and the growth phase of the trial lasted for 84 days. Briefly, fish were fed with two isoenergetic and isonitrogenous experimental diets in triplicate (3 tanks/diet). A control diet containing only fish and plant proteins (CTRL), and an insect diet containing 10% of *Hermetia illuncens* meal (Hi10) were tested in rainbow trout (*Oncorhynchus mykiss*). At the end of feeding trial two fish per tank were sampled (6 fish/diet) and the whole intestine was aseptically removed. The fecal matter containing transient (allochthonous) intestinal bacteria were collected and the resident (autochthonous) microbiota was obtained by scraping the intestinal mucosa of whole gut length and excluding pyloric caeca.

Bacterial DNA extraction and sequencing: DNA was extracted from three samples per feed and six biologic samples of feces-mucosa per each dietary fish group. For the characterization of microbial communities, the V4 region of the 16S ribosomal RNA gene was amplified, using the oligonucleotides:

515F: 5′-GTGYCAGCMGCCGCGGTAA-3’ | 806R: 5′-GGACTACNVGGTWTCTAAT-3 ′

The amplicon library preparation and sequencing were carried out using an Illumina MiSeq platform and protocol (#15044223 rev. B). The raw sequencing data were analyzed with QIIME 2™ (v. 2018.4) pipeline at the default setting (*Bolyen et al*., 2018). The Silva database was used for the recognition and taxonomic classification of the sequences obtained. The taxonomical classification was performed down to the genus level. OTUs (Operational Taxonomical Unit) assigned to chloroplasts and mitochondria were removed from the analysis as of eukaryotic origin. The relative abundance (%) of each bacterial taxon was determined for every single sample (fish or feed) and for every experimental group (CTRL and Hi 10%). Starting from sequencing data processed by the QIIME pipeline the relative abundance of each OTU was calculated. Alpha (within a single sample) and beta diversity (between samples) have been performed using QIIME alpha-phylogenetic and beta-phylogenetic commands. The “observed OTUs”, “Shannon”, “Faith-PD”, and “Evenness” alpha diversity indices were calculated at the same level of rarefaction, i.e. considering the sample size with a lower number of sequences. Beta diversity was calculated using both the weighted (presence /absence/abundance) and unweighted (presence/absence) UniFrac distance matrix. The UniFrac distances between the microbial communities of the individual samples were visualized by means of two-dimensional scatter plots (PCoA).

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Results

Metabarcoding analysis of feed-associated bacteria showed that the microbial communities of CTRL feed differed from those of experimental feed Hi10. The microbial community structure for each feed was outlined at the phylum, class, order, family, and genus. The microbiome profile of all samples comprised 6 different phyla, 11 classes, 17 orders, 25 families, and 30 genera. The number of reads taxonomically classified according to SILVA database was 168,562. Six hundred and thirteen OTUs at 99% identity were identified in feed samples and classified into 134 different taxa. Good's coverage in all samples reached a value of 0.99, indicating that sequencing coverage was achieved and that the OTUs found were representative of intestinal microbial communities. To elaborate on alpha rarefaction analysis, samples were normalized at a sequencing depth of 3,900 reads. At the order level fish fed diet containing 10% of insect meal showed a significantly higher relative abundance of Bacillales (34%) in their intestinal microflora compared to the control group (1.7%). This important enrichment in Bacillales was due to an increased number of bacteria belonging mainly to Bacillaceae (22%) families in the intestine of fish fed Hi10 diet. Accordingly, at genus taxonomic level, Bacillus, Oceanobacillus, Virgibacillus, and Paenibacillus Corynebacterium were more abundant in Hi10 samples, while fish fed control diet showed higher percentage of Shigella and Shewanella genera, the last one practically absent in fish fed Hi10 diet.

Conclusions

In agreement with the majority of studies on the partial substitution of fishmeal with insect meal (*Terova et al*., 2019; *Rimoldi et al*., 2019; *Bruni et al*., 2018; *Antonopoulou et al*., 2019; *Huyben et al*., 2019), the experimental diet with 10% *Hermetia illucens* (Hi10) had an important effect in modulating the intestinal microbial communities of trout. In particular, Hi10 diet increased gut microbial richness (Observed OTUs index) and numerically the diversity (Shannon diversity index p=0.05) as compared to control fish. In general, high gut microbial richness and diversity are considered desired features because they are usually associated with the health status of the host. Based on the ratio of Firmicutes:Proteobacteria, fish fed Hi10 diet showed a dominance of Firmicutes that has been associated with dietary plant ingredients that are rich in fibers, whereas an animal protein-based diet usually favors the dominance of Proteobacteria (*Terova et al*., 2019; *Ingerslev et al*., 2014 a; 2014b). Dietary inclusion of insect meal influenced the microbial intestinal profiles of trout both qualitatively and quantitatively. Indeed, multivariate analysis of bacterial communities revealed a significant relationship between diet and microbiota associated with fish intestines.

A REGIONAL MULTI-TROPHIC AQUACULTURE ECONOMIC MODEL

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In the past decades integrated multi-trophic aquaculture (IMTA) systems have been studied in several countries, potentially posing a more sustainable alternative to traditional mono-culture aquaculture. IMTA production systems involve species from different trophic levels, where the dissolved and particulate effluent produced by one species can be partially reduced through the cultivation of extracting species, such as filter feeders and seaweed (Neori *et al*., 2007). However, the many challenges related to the production of multiple species in the same operation have hampered implementation by the finfish producing industry.

Finfish farming typically occurs in arrays of suspended net pens with free water exchange ensuring the supply of oxygenated water and waste removal from the pens. The farming operations are complex, relying heavily on service ships for stocking, harvesting, cage assembly and maintenance. This means that installing structures for seaweed or shellfish adjacent to the net pens, would represent an obstacle for the day-to-day functioning of the fish farm. Additionally, regulatory constraints do not permit the installation of IMTA in relevant salmon producing countries, such as Norway. Whereas in Chile, economic considerations have hindered the adoption of IMTAs at a commercial scale (Buschmann *et al*., 2009; Ellis & Tiller, 2019).

To advance the implementation of IMTA the concept should be considered on a regional level instead of farm level (Chopin, 2017, Ellis & Tiller, 2019, Sanz-Lazaro & Sanchez-Jerez, 2020). An integrated coastal area management (ICAM) approach was proposed by Chopin (2017), with several strategies to recover nutrients. This could help untangling some of the complexities of operating IMTAs, potentially increasing adoption from the industry.

The aquaculture industry of the Faroe Islands is largely dominated by the production of Atlantic salmon (*Salmo salar*) but in the last decade, sugar kelp (*Saccharina latissima*) has been farmed in the fjords of the Archipelago. Studies carried out in the EU H2020 AquaVitae project, investigating the direct assimilation of fish farm waste by blue mussels at a commercial fish farm have shown limited effect (á Norði, 2022). However, an ICAM approach to multitrophic aquaculture can potentially limit the accumulation of dissolved and particulate effluent in the fjord, thus increasing the interest from the industry on adopting this novel strategy. Multi-trophic aquaculture economic performance is usually contrasted with monoculture, for increased acceptance, it must appear profitable (Lance et al., 2017).

Economic analyses have been developed for similar models in Canada (Carras *et al*., 2020), but financial performance and markets vary greatly between countries. The aim of this paper is to advance the knowledge on the economic feasibility of producing three ecosystem-balancing species at different trophic levels in a regional environment by introducing an economic model based on three species; the already established aquaculture operations of salmon and sugar kelp in the Faroe Islands, as well as the cultivation of blue mussels *(Mytilus edulis)*, as filter feeders to complete the system. In the Faroe Islands, the potential for establishing multi-trophic aquaculture has increased with the recent changes to aquaculture legislation which now allow farming of multiple species in the same fjord. Therefore, the model applied to the Faroese context can provide important insights to support the adoption of multi-trophic aquaculture in larger salmon-producing countries, such as Norway and Chile.

The value-chain model employed to assess the economic feasibility will take into account commercialisation and market dynamics. Profitability will be estimated using net present value and market appraisal, and the analysis will also include sensitivity analysis and risk assessment.

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TECHNOLOGICAL SOLUTIONS FOR REDUCING MICRO- AND MACRO- PLASTIC FROM FISH FARMING

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Introduction

Plastic is extensively used in aquaculture. Its strength, durability, flexibility, tensile and waterproof properties, in addition to the low cost of this material, make plastic optimal for diverse applications in the industry. However, Scale Aquaculture, as a technological supplier, recognise that marine litter, and especially plastic pollution, is a major environmental problem. It is important for us to work with reducing emissions of plastic in nature and increasing the recycling of our products.

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 (Hognes and Skaar, 2017). There is a need to increase the recycling rate of the plastic equipment and derive the most value from resources during their lifetime Additionally, the Directive (EU) 2019/904 on the reduction of the impact of certain plastic products on the environment includes i.a. requirements for producer responsibility schemes for certain plastic products, including equipment from fisheries and aquaculture industry. The directive requires equipment suppliers to have solutions for collection, reuse and recycling of plastic equipment.

Lost equipment as consequence of storms, wear and tear of plastic materials used in off-shore farms generate large amounts of plastic waste to the surrounding regions of the farm (Lusher et al., 2017). Plastic waste from fish farming has been found along the Norwegian coast. Ropes were the major polluting materials, but parts of other plastic equipment such as feeding pipes, walkways, and floating collars were also found (Vangelsten et al., 2019). In addition, weathering of plastic equipment at the fish farms can release microplastics and contribute, to another extent, to the microplastic pollution in the marine environment. Recent studies have estimated the amount of microplastics released from materials used in fish farming. In 2020, a study assessed the abrasion of feeding pipes caused by the fish feed pellets to analyse the amount of microplastics released (Gomiero et al., 2020). Results showed that aged and curved pipes had higher abrasion rates compared to new and straight pipes. Aged pipes released 2 to 3 times more microplastics than new pipes. In average, abrasion of the feeding pipes was estimated to release 0.24 g of microplastics per meter of feeding pipe and per day. Some reports estimate that up to 200 tons of microplastics per year are release from feeding pipes (Vangelsten et al., 2019).

Small microplastics (< 50 µm) can affect marine organisms like reduced food intake, delayed growth, and decreased efficiency of the immune system of the organisms affected (Abihssira-García et al., 2020). As for other equipment used in fish farming, there is no detailed information about degradation rates or microplastic release as far as we know. Though, it has been roughly estimated that the degradation rates of plastic in the Norwegian marine environment is less than 0.5% per year (Booth et al., 2017).

We will present the work that Scale Aquaculture is doing regards to reduce emissions of plastic in nature from fish farms both regards to micro- and macro-plastic. We will give examples on possible technological solutions. Results from an ongoing project called Circular plastic products for sustainable aquaculture ("SPARE") will also be presented.

Results

The main objective of the project "SPARE" is to increase the proportion of recycled plastic in several of our products used in the aquaculture industry. In 2021, we mapped out the material flows of plastics in the company's value chain, all the way to post-use in the fish farm and subsequent recycling. Our estimates show that we currently recycle around 42% of our plastic equipment, an amount we want to increase. As an extension of this study, we conducted a pilot project where we collect one discarded floating collar and produced 17 tons of recycled plastic pellets. After grinding and extrusion, the re-granulated was analysed and characterized to find the most suitable area of application. Relevant analysis like strength calculations, durability, tensile strength was carried out. We are now using these recycled plastic pellets to produce new plastic-based equipment for the aquaculture industry. Testing and documentation of full-scale pilot installation of selected products made from recycled material is now being carried out.

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In the aquaculture industry, air-based feeding is typically used, but during the last years water-based feeding is becoming more common. Water-based feeding systems represent a key solution for the reduction of microplastic emissions. At the end of 2021, we developed and launched our new barge with subsea/ water-based feeding technology. After testing subsea/ water feeding through pilots in both Tasmania and Norway, we experienced promising results on several parameters such as no microplastic emissions, less noise, reduced energy consumption and increased feeding capacity.

A more detailed presentations of the results will be given.

Acknowledgement
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AN INTERGRATED MULTITROPHIC AQUACULTURE IN GREECE (EASTERN MEDITERRANEAN): CO-CULTURE OF FISH AND BIVALVES

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Introduction

During the past 50 years, mariculture has become one of the most productive industries worldwide. The most known effect of a typical aquaculture is the releasing of dissolved nutrients and particulate organic matter in the water column as well as sediment organic enrichment causing biological and geochemical changes. Integrated multitrophic aquaculture (IMTA) has been suggested as an innovative method that ensures sustainable development in agreement with the EU directions for Blue Growth and Blue Economy. It is defined as the cultivation of two or more aquatic species from different trophic levels in the same area in order to mimic the energy flow in natural ecosystems (Chopin et al., 2004). IMTA organisms may include bivalves and echinoderms that consume phytoplankton and particulate organic matter. In this study, the Mediterranean mussel (*Mytilus galloprovincialis)* and rayed pearl oyster (*Pictada imbricata radiata*) were co-cultured with fish (*Sparus aurata*). Mediterranean mussels and pearl oysters are filter-feeding organisms that feed on phytoplankton and selected particles retained after filtering the water column (Bayne, 2002). The primary objective of this study was to develop IMTA methodology suitable for use in the oligotrophic eastern Mediterranean focusing on the survival, robustness, and growth of the above mentioned species. The results can be used as a basis for setting up large scale commercial IMTA systems in Greece.

Materials and Methods

The IMTA trials were implemented in three different fish farms in the Aegean Sea with similar cultivation methods, but different eutrophic conditions. Characterisation was based on Eutrophic Index (Primpas et al., 2010). The first aquaculture farm (Aq1) was characterized as higher mesotrophic, the second farm (Aq2) as lower mesotrophic and the third (AQ3) as oligotrophic. The same IMTA cultivation method was applied in all farms.

For IMTA mussel cultivation, the traditional longline technique was used: Mussel seed (spat) was collected by hand either from hard surfaces within the farm area and put in longlines which were then submerged around the middle cage in the centre of each fish farm. The cultivation period was nine months, which is when they reached marketable size (5–6 cm) from an initial 1.5 ± 0.5 cm. Mediterranean mussels were not placed in Aq3 as they were not locally available. To estimate the growth of the mussels, 30 individuals were randomly selected from different parts of the longline and were measured on October, February, May, and June (2020-21) for total weight, as well as shell and flesh morphometrics. The measurements were used to calculate the Condition Index, Specific Growth Rate and Meat Yield (MY) (Theodorou et al., 2021). To determine Mediterranean mussel growth, our data from IMTA-mussels were compared with data from Keskin and Ekici (2021), who provide data from typical mussel farms in eastern Mediterranean.

Pearl oysters were cultivated in baskets fabricated by SEAPA© for oyster growers. Spat was again collected from natural populations in the vicinity of each fish farm. Before putting them in the IMTA system, the pearl oysters were divided into three different size classes (40 in each class) and placed in separate baskets with holes of increasing diameter. Oyster baskets were deployed around the middle fish cage in the centre of each fish farm at a depth of 3 metres for 9 months in total in all three farms. All measurements and metrics taken for mussels were also taken for pearl oysters. CI and MY data was used from Theodorou et al. (2021) to compare IMTA growth to natural populations (since pearl oyster cultivation is not yet allowed in Greece).

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Results

In Aq1, which had the highest eutrophic conditions mussels grew with an SGR of 0.4% per day to a 21.2 g. In Aq2 the grew with an SGR of 1.3% to 16 g over the 9 month period. Additionally, the CI was 42% and MY 19% in Aq1 while CI was 33% and MY 35% in Aq2. Based on the bibliographic data the CI of typical mussel farm was 35% and the MY 10%. CI comparison between the two farms showed that Aq1 had a significantly higher CI in February, May and June (ANOVA $F = 38.408$, $p<0.05$).

Pearl oysters also showed positive SGR in all the study sites. In Aq1 the SGR was 0.7% per day, in Aq2 it reached 0.9% per day, and in the most oligotrophic Aq3, it was found to be 0.4 per day. Within 9 months, in Aq1, pearl oysters grew from 6.6 g to 39.5 g, in Aq2 from 4.4 g to 48.9 g, and in Aq3 from 7.2 g to 25.5 g. CI was 53% in Aq1, 56% in Aq2, and 19% in Aq3. Bibliographic data showed that the CI of natural populations was 30% to 45%. MY was 26% for Aq1, 33% for Aq2 and 27% for Aq3, while natural MY ranged from 20 to 31%.

Discussion

The first IMTA bivalve pilot cultivation in the eastern Mediterranean was successful. The Mediterranean mussel was able to successfully grow in IMTA conditions, but not in all waters; the Mediterranean mussels have not colonized oligotrophic waters being unable to assimilate adequate food resources to ensure the survival of their population. The pearl oysters, on the other hand, is a species highly suitable for IMTA cultivation because it can grow successfully in environments with very different trophic statuses. The culture methodology applied in an IMTA system is similar to techniques used in traditional monoculture bivalve culture. This is very important since cultivation equipment and know-how is already available to fish farmers and can be easily applied.

Comparing the values of CI and MY, the IMTA mussels had higher CI and MY than reported values for traditionally farmed mussels from the North Aegean. These results indicate that when Mediterranean mussels grow in proximity to multiculture farms, they have more flesh in their shell in comparison to those growing in a typical mussel farm. As opposed to Mediterranean mussels, oysters were successfully cultivated in all locations: they grew properly at commercial size at all sites with all types of trophic conditions despite their high requirements for phytoplankton biomass. However, they they exhibited a different growth rate. They reached commercial size (5–6 cm) within a few months in Aq1 and Aq2, but in the oligotrophic aquaculture (Aq3) they needed nearly a year. In conclusion, IMTA is a dynamic system that can be adapted to the conditions prevailing in each region. Depending on the water variables, IMTA may involve cultivating different types of organisms near the fish cages.

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APPLICATION OF FECAL CORTICOID METABOLITES (FCM) AS STRESS INDICATOR DURING PARR-SMOLT TRANSFORMATION FOR ATLANTIC SALMON (*Salmon salar L.*)

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Introduction

Traditionally, farmed Atlantic salmon stay in open net-pens throughout their sea-cage production cycle. Farmed salmonids are more frequently exposed to stressful events, such as poor environmental conditions and farm operations (e.g., sea lice treatment, splitting), which could deteriorate the well-being of fish. Poor welfare can result in low growth rate, immunosuppression, and high mortality in farmed salmon (Oliveira et al., 2021). Therefore, assessing the severity of stressors helps not only to prevent fish from poor welfare conditions, but also to improve farm operations. Plasma cortisol (PC) level is widely used as the acute stress indicator, since it increases significantly in response to different physical, chemical, and biological stressors. PC levels start to increase several minutes after fish are subjected to a stressor. Fish were sampled with large dip nets or with nets weighted bottom ropes from under commercial production cages. Capture effects are hardly avoided, and the baseline level of PC is more difficult to estimate, since fish may stay in stressful situations for several minutes before they can be anesthetized, and blood samples can be taken. Other matrices for cortisol measurements are being developed, to minimize interference from sampling procedures themselves and extend valid sampling time of using cortisol level as a stress indicator in the aquaculture industry. Based on cortisol metabolic pathway, fecal corticoid metabolites (FCMs) to monitor stress level of fish with less interference from sampling procedures is currently under development. We aim to compare PC and FCM under stress situations and the effect of sampling procedures on PC and FCM levels. We hypothesize that FCM level is in correlation with PC level and that sampling itself has less effect on FCMs level than on PC level.

Materials and methods

The experiment was conducted at the Industrial and Aquatic Laboratory (ILAB) in Bergen, Norway. After two weeks of acclimation, the salmon started its parr-smolt transformation, which was used as the stressor in this experiment. To measure cortisol stress response to the parr-smolt transformation, fish were sampled before (C1), during (S1), one week (S2) and five weeks (C2) after transfer to seawater. 18 fish were sampled from three replicate tanks. Three fish were sampled each time, and the time interval between each sampling being around 40 minutes. Blood samples were collected from the caudal vein using EDTA (ethylenediaminetetraacetic acid) vacutainers (VACUETTE® TUBE, Greiner bio-one, Austria), centrifuged for 10 min at 3040 g, and plasma was removed and stored at −80 °C until cortisol analysis. Intestinal contents were collected as feces samples in this experiment to ensure enough material for analysis. Samples were stored at −80 °C until analysis. Cortisol concentration in plasma and FCM level were measured by using a commercial EIA kit (Cortisol ELISA KIT; Neogen® Corporation, KY, USA). Data analysis was conducted in R software™ version 4.0.4. A *p*-value below 0.05 was considered statistically significant.

Results

Statistically significant increases were found between C1 and S1, C1 and S2 for both PC and FCM. For FCM. There was a significant decrease of FCM level from S1 to S2, and no statically significant difference was found between S2 and C2. The level of PC in S2 was lower than S1 ($P > 0.05$) and significantly higher than C2. No differences were found between C1 and C2 for both PC and FCM. Sampling time and stressor had statistically significant effects on both PC and FCM level. Although cortisol levels of the second sampling point were higher than the ones of the first sampling point in all samples, statistically significant differences between two sampling points were found in the C2 group for both PC and FCM, and in the C1 group only for FCM. Significantly positive correlations were identified between plasma cortisol and FCM (Spearman's $p < 0.05$) in all samples.

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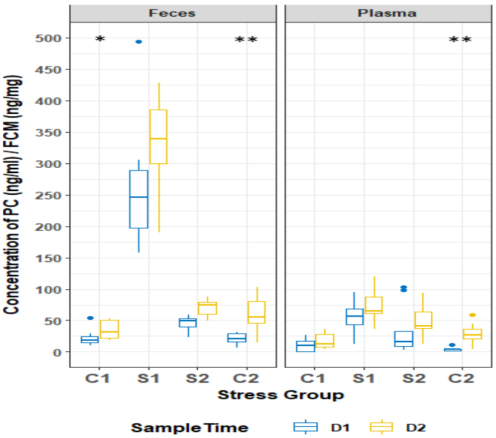

Figure 1.Concentration of PC and FCM in four sampling groups, each sampling group has two sampling time points. Asterisks indicate the degree of significance (: p <= 0.05, **: p <= 0.01) as assessed by Wilcoxon test.*

Discussion

The observation of changes in PC during parr-smolt transformation is in accordance with the regular patterns of cortisol responses in Atlantic salmon. The PC level usually stays elevated during and after parr-smolt transformation and only declines to the baseline level after the fish has stayed in sea water for about one month (Sundell et al., 2003). The 40 minutes interval between two sampling points could explain the significant increase of PC by the second sampling points. Normally, the concentration PC in Atlantic salmon reaches peak levels around 30 mins after an acute stress exposure (Fast et al., 2008). More stable values of FCM between two sampling points were expected. Because radiometabolism studies in domestic livestock demonstrated that there is a time lag between provoking stress responses and FCM, which is related to the gut passage time(Palme et al., 1996). In this experiment, the contents were collected from mid and distal intestine and fish is relatively small (wet weight around was 44.3 grams in the C1), which could shorter the time lag. Further study of the time course and FCM dynamics in the Atlantic salmon post stress exposure are essential to evaluate stress events and farm operations in aquaculture industry. To be noted, two control groups (C1 and C2) without stressors had higher increase-rates between two sampling points than their stressful groups for both PC and FCM. These findings together with recent studies, indicate that conducting a novel acute stress challenge to the fish can differentiate whether fish is chronically stressed. Significant correlation between PC and FCM were demonstrated in all samples, which indicates FCM could be used an alternative matrix to PC for measuring cortisol acute stress responses in Atlantic salmon. The results draw researchers' attention to the importance of standard sampling procedures and experimental designs to the robust and repeatable results

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PHYTOBLOOM© ELITE FORMULA: DEVELOPMENT OF A PREMIUM MICROALGAE DIET FOR ROTIFERS NUTRITION

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Introduction

The culture of rotifers (*Brachionus plicatillis*) is essential for successful marine larviculture, since altricial larvae require live feed during the first developmental stages. Despite the fact that rotifers are cultured for decades, its cultivation still presents relevant challenges. Rotifers full nutritional requirements and metabolism are still poorly understood, which difficults the dietary optimization of this cryptic species. Rotifers are microalgae grazers and *Nannochloropsis oceanica* is particularly successful for feeding *Brachionus plicatilis*. The expansion of aquaculture is rapidly leading to the reduction of labour intensive and costly activities such as microalgae production. The substitution of live microalgae to industrially produced microalgae products is the solution for this challenge. Within the available microalgae products (paste, powder, liquid concentrate), the liquid concentrates have become popular due to their easy usage, since they do not require *in situ* daily preparation steps (e.g. hydration, blending). Most of the commercially available microalgae products require improvements for aquaculture specific applications, such as the nutrition of rotifers. Some of the most relevant challenges in industrially produced *Nannochloropsis* sp. are agglomeration, decantation and bacterial load. The bacterial load can have negative impacts not only on rotifers culture but also larviculture (Turgay et al., 2020), particularly in species with susceptible larval stages. The objective of this work was to develop a premium microalgae diet for rotifers nutrition, with improved microbiological quality and rotifers growth efficiency.

Material and methods

The production of Phytobloom© ELITE formula (ELITE), *Nannochloropsis oceanica* liquid concentrate, was performed with an improved saline diluent (200ppt) to achieve 18% of dry weight. For this premium product a selection criterion of the batches of industrially produced microalgae according to bacteria load was applied. The comparison of the quality and efficiency of ELITE (n=2 batches) was performed in comparison with two microalgae commercial diets (CP1 and CP2) (1 batch of each product) commonly used in rotifers nutrition. Products quality was analysed according to the total bacteria detected through PCA, TSA and TCBS media, presence of ammonium, nitrates, pigments (chlorophyl a and total carotenoids) and microalgae agglomerates presence and dimension. All products were used in rotifers culture under batch (4 days) production system (ELITE n=13, CP1 n=40, CP2 n=4). Rotifers were cultured in cylindroconical tanks (1.2m^3), with oxygen injection and aeration with an initial concentration of 500 rotifers/ml. The water quality was daily monitored and maintained at 28ºC, pH 7.5-8, ammonia below 1mg/L. The dosage of ELITE in the first day was 3.2ml of product per million of rotifers and 2.5ml per million of rotifers in the remaining days. The recommended feeding protocol of the commercial products was applied in the culture. Rotifers concentration and percentage of rotifers with eggs were evaluated daily.

Results and Discussion

ELITE is a new formula product presented significantly lower total bacteria content in comparison to the other rotifers diets (Figure 1A). The use of an optimized saline diluent promoted a reduction of the microalgae agglomeration and dimension of the microalgae agglomerates (Figure 1B). The new productELITE significantly improved rotifers growth under batch system in comparison to the two commercial products tested (Figure 1C).

The reduction of the number and size of the particles in the water column can promote the reduction of the microalgae decantation, thus improving particle suspension on the water column. These characteristics lead to an increase of the availability of microalgae for rotifers nutrition, reduces waste formation and toxic ammonia accumulation in the tanks. The reduction of the bacteria load in the product through the new saline diluent used in the liquid concentrate, along with the selection of batches with lower microbial loads allowed the improvement of the new product microbiological quality. Altogether, these characteristics promoted significant improvements on rotifers growth performance when compared to the other tested commercial products. In conclusion, the new premium diet ELITE developed for rotifers nutrition successfully improved rotifers production under batch production mode in comparison to other commercial diets commonly used in rotifers culture.

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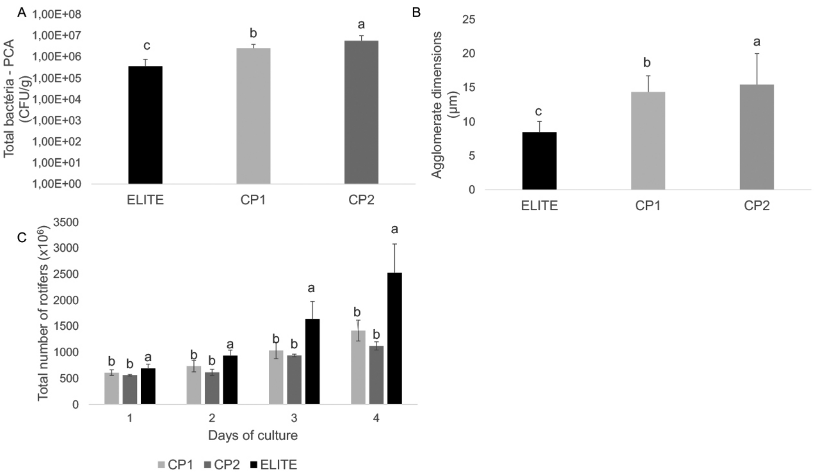

Fig 1 – Rotifers new diet ELITE and commercial diet 1 and 2 (CP1 and CP2) quality characterization: A) Products total bacteria analyzed through PCA media, B) Microalgae agglomerates dimensions and C) Total number of rotifers produced per day of batch. Data is expressed as means and standard deviation and significant differences between treatments are represented by different letters (One-way ANOVA, post hoc Tuckey, $p<0.05$).

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PERCH RHABDOVIRUS IN PIKE PERCH (*Sander lucioperca*) AND EUROPEAN PERCH (*Perca fluviatilis*) FROM SWISS AQUACULTURE FACILITIES

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Introduction

Perch rhabdovirus (PRV) can lead to high mortality in European perch (*Perca fluviatilis*) and pikeperch (*Sander lucioperca*). Larvae and juveniles are mostly affected. Typical clinical signs are spiral swimming movements and, later in the disease course, cachexia due to emaciation. Driven by intensive fish trade, different variants of PRV are currently circulating in European percid aquaculture.

PRV belongs to the *Perhabdovirus* genus. The perhabdovirus genome is made of a linear, negative-sense, single-stranded RNA, which codes for 5 proteins. Differences in the nucleoprotein (N) and phosphoprotein (P) genes are used to differentiate genetically close viruses (Bigarré et al. 2017). A total of four PRV infections in pikeperch and European perch are described in this report.

Material & methods

The first submission involved an import of pikeperch from France that was routinely examined after arrival in the quarantine. The second case concerned pikeperch from the same facility as the first submission, which were analysed due to clinical symptoms and low mortality. In the third investigation, a pikeperch spawner from another facility was sent for routine inspection. Finally, in the fourth case, European perch were sent for diagnostics due to clinical symptoms and increased mortality.

All animals were subjected to pathological examination and tested for the presence of PRV using conventional RT-PCR (Pallandre et al. 2020) followed by sequencing. For phylogenetic analysis, the total RNAs were sent to the laboratory of Ploufragan-Plouzané-Niort, ANSES, Plouzané, France. A new nested PCR-based method was used to amplify and sequence the complete N and P genes (Pallandre et al. 2022).

Results

Using a range of cPCR, PRV was detected in all four groups. Interestingly, the pikeperch that were examined for control purposes after importation, as well as the spawner, showed no clinical signs of PRV infection. The pikeperch sent for diagnostics due to low mortality presented few symptoms such as spiral swimming movements and lethargy. However, the animals recovered after few days and no further mortality occurred. In contrast, the European perch showed severe clinical signs, particularly spiral swimming, and high mortality. These animals were euthanized for welfare reasons.

The viruses from the asymptomatic pikeperch (quarantine, spawner) were almost identical to another virus isolated in Belgium in 2016. The virus from the pikeperch presenting low clinical symptoms was more closely related to a subgroup of viruses of different origins (France and Belgium). The rhabdoviruses found in the diseased European perch were more closely related to a subgroup of viruses isolated in France.

Discussion

This study shows that PRV is more widespread in percid aquaculture than previously assumed. The pikeperch examined in this study showed significantly less clinical symptoms than the European perch. However, the virus was also detectable in these clinically mostly inconspicuous pikeperch. The viruses detected in this study in the pikeperch cluster together with PRVs found previously in pikeperch. The viruses found by the European perch are also related to other isolates from European perch. However, all viruses documented in this report seem different from the rhabdovirus isolated in Switzerland in 2013 and the Egli virus detected in a Swiss aquaculture in 2017.

Systematic phylogenetic studies will help in future to obtain more information on epidemiology of the virus and thus optimise control mechanisms to prevent further spread of the disease.

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SUPPLEMENTATION OF COMMERCIAL FEED WITH VITAMINS AND IMMUNOSTIMULANTS TO INCREASE IMMUNITY OF EUROPEAN PERCH (*Perca fluviatilis*) AND AVOID SECONDARY INFECTIONS AFTER STRESS SITUATIONS

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Introduction

European perch (*Perca fluviatilis*) is a native fish species, which is highly appreciated for its meat in Switzerland. However, most of the perch consumed in Switzerland originates from wild populations in Eastern Europe. To reduce (over)fishing and to increase the amount of locally produced aquaculture fish, the company Valperca SA started production of perch in 2007. The company is a pioneer in Swiss perch production and even worldwide. Perch aquaculture is still a relatively new branch of production. Consequently, knowledge about this fish species' needs under production conditions is still limited, including knowledge about stress-related secondary diseases. Currently, increased stress-induced vulnerability and subsequently increased disease susceptibility or even mortality due to secondary bacterial infections after transport or fish grading is one of the main concerns in perch production.

Aquavac Ergosan® is made of two brown seaweed species, *Laminaria digitate* and *Ascophyllum nodosum*. Many in vitro and in vivo studies (on various species, particularly salmonids and sturgeons) have shown the benefits of Ergosan® on fish growth, survival rate, immunity and broodstock performance. Vitamins, especially vitamin A, D, E & C, are also known as immunoregulators and immunostimulants, tested in farm animals, inclusive aquaculture animals (e.g. salmonids, cyprinids, tilapia). Both, the use of Aquavac Ergosan® and of the abovementioned vitamins have not yet been tested on perch. Immunostimulation is especially important during stress conditions, like shortly before, during and after transport, vaccination or grading of fish. At these time points, perch are especially vulnerable to bacterial infections with *Flavobacterium* sp. causing disease and mortality.

Material & methods

Perch (*Perca fluviatilis*) of 10g (approximately 100 days post hatching) will be bath challenged with *Flavobacterium columnare*. The bacterial strain used originates from a previous disease outbreak in a warm water recirculating system. The amount of bacteria required for this challenge will be determined in a preliminary pilot trial performed on a reduced number of fish (six groups of 25 fish each). In order to be able to differentiate effects of the different feed supplements, the infection should cause low to moderate mortality (approximatively 25%) in the infected groups.

The main trial will include four groups:

- Group 1: Feeding with commercial feed without supplementation (control group)
- Group 2: Feeding with commercial feed supplemented with Aquavac Ergosan®
- Group 3: Feeding with commercial feed supplemented with vitamins A, D, E & C
- Group 4: Feeding with commercial feed supplemented with Aquavac Ergosan® and vitamins A, D, E & C

All groups will be exposed to two different treatments in triplicates of 27 fish each:

- Treatment 1: The fish will be netted and subjected to two times 30s out of the water to simulate husbandry related stress (mechanical stressor).
- Treatment 2: The fish will be netted and subjected to two times 30s out of the water to simulate husbandry related stress (mechanical stressor), followed by infection by immersion with *Flavobacterium columnare*.

Mortality, clinical symptoms, histopathology and differences in expression of immune response parameters (interleukin-1β, interleukin-8 and tumor necrosis factor α 2) will be assessed.

The aim of the study will be to compare the benefit of supplementation of commercial feed with (1) Aquavac Ergosan®, and (2) with vitamins A, C, D and E to increase immune capacity of aquaculture perch. Boosting fish immune system should reduce secondary bacterial infection after husbandry related stress conditions.

BIOTECHNOLOGICAL POTENTIAL OF MICROALGAE AND PROBIOTIC STRAINS IN A CO-CULTIVATING SYSTEM

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Introduction

The divergence of microalgae species is a rich source of many different metabolites with bioactivity. Many of the compounds extracted from microalgae biomass show beneficial effects on health. Recently, co-cultivation of microalgae and bacteria have emerged as a promising solution that can enhance synthesis of active compounds and induce it with new compounds not specific to the monoculture. Rich sources of nutrients like probiotics and microalgae biomass serve as beneficial food supplementation as their influence on diets is the focus of scientists. These microorganisms and their extracts have great potential to boost health and improve one's well-being. Algae are considered as prebiotics and most studies implement them as enhancers to the probiotics performance. However, a different focus to switch their roles in co-cultivation is approached. Several studies have shown that the addition of algae, along with probiotics, can influence the microbiota, and improve gut health and overall yield in fish and shrimp aquaculture. This research focused on further enhancing their benefits by combining them in a co-culture manner which showed promising results.

Materials and methods

The co-cultivation system was established with microalgae *Chlorella vulgaris* and the probiotic species *Lactobacillus plantarum*.Optimization of the inoculum ratio was done in 24-well plates which served as a base for further „scale-up“. The „scale-up“ of the co-culture system was performed in flasks for 5 days as batch cultivation with Tryptic soy broth as medium. The flasks were shaken on an orbital agitator at 125 rpm and were illuminated with warm white LED lights (2700K). To determine the bioactivity of their co-culture co-existence, a range of biological assays were performed. These analyses were performed on the biomass of the monocultures and that of the co-culture. The lipid composition was analyzed by fluorescent microscopy using Nile Red staining method. Pigment composition such as chlorophylls, pheophytins and total carotenoids of *C. vulgaris* in the monoculture was compared with the composition in the co-culture system. The potential toxicity of tested samples was determined by conducting a zebrafish *Danio rerio* embryotoxicity test (OECD 236, 2013). Metabolite products synthesized in the monoculture as well as in the co-culture biomass were analysed using quadrupole time-of-flight (q-TOF) mass spectrometry revealing the production of new compounds.

Results

Optimization of the inoculum ratio showed that the best ratio was in favor of the microalgae *C. vulgaris* with a cell number of 10^7 cells/mL while the cell number of *L. plantarum* was 10^5 CFU. Nile red staining revealed that the growth conditions were not stressful for the microbes in the co-culture (Figure 1). Chlorophyll a, b and total carotenoid compounds increased in their value through the cultivation of the monoculture and decreased in the *C. vulgaris* co-culture(Figure 2). Evaluation of the embryotoxicity of *C. vulgaris* in zebrafish *D. rerio* showed no negative impact on survival and development below the concentration of 0.025 mg/mL for *C. vulgaris*. For the monoculture of *L. plantarum* as well as the co-culture, no toxicity was obeserved at concentrations below 6.25 µg/mL. Q-TOF analysis revealed the presence of molecules with already proven antihypercholesterolemic and antiobesity activities.

Discussion

From the results shown in the previous section we can conclude that the coexistence of *C. vulgaris* and *L. plantarum* in the same medium, as well as the co-cultivation conditions, were optimal and enhanced bioactive compound synthesis. Furthermore, the co-culture system was succesfully „Scaled up“ and the parameters used for this research were not stressful for the co-culture. It should be noted that Q-TOF analysis showed many bioactive metabolites which could be essential for a balanced diet. These positive bioactive potentials and comprehensive analysis serve as a base for further implementation of the co-cultured biomass into aquaculture systems.

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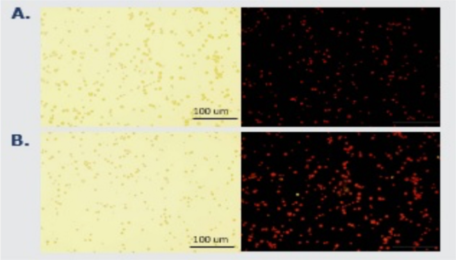

Figure 1: **A.** Microscopic view of *C. vulgaris (left image) and C. vulgaris stained with* Nile Red (right image) visualized under the fluorescent microscope. **B.** Microscopic view of *C. vulgaris and L. plantarum co-culture*(left), *C.vulgaris and L. plantarum co-culture stained with* Nile Red (right image). Red-colored cells show polar lipids, while yellow-colored cells showed the content of neutral lipids in co—culture system.

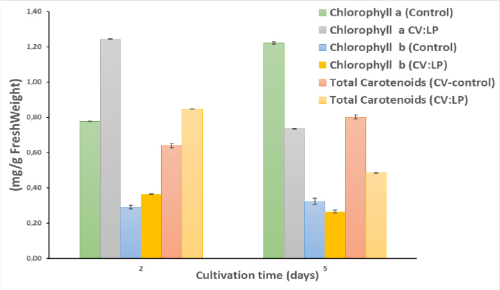

Figure 2: Comparison of chlorophyll a, chlorophyll b and total carotenoids in *C. vulgaris* (control) and *C. vulgaris* (Co-culture: „CV:LP”).

Reference

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AQUAVIP (AQUACULTURE VIRTUAL CAREER DEVELOPMENT PLATFORM FOR THE SOUTH BALTIC REGION) SUCCESS AND FURTHER POTENTIAL

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Introduction

After almost three years of carrying out AquaVIP – Aquaculture Virtual Career Development Platform for the South Baltic Region project, led by Klaipeda Science and Technology Park, accompanied by University of Rostock, University of Gdańsk and Klaipeda University, the partners and stakeholders along the aquaculture value chain share a great success in fostering human resources capacity through cross-border training and networking to meet the objective and boost aquaculture labor market within the South Baltic region. Recognizing the significant demand for aquaculture professionals and a need for progress in innovative technologies in the South Baltic Region AquaVIP project focused mainly on: investigating best practices, testing innovative methods and tools, exchanging knowledge and experience related to human resource capacities for the aquaculture sector, training students and professionals in innovative aquaculture methods, cooperation and networking with organizations pursuing the same mission, and promotion of sustainable and innovative solutions.

Materials and methods

AquaVIP project offers have been carried out through four pillar services, namely: AquaVIP experiments, AquaYouth, AquaProfi, and AquaTION.

AquaVIP experiments performed in partners' facilities foreseen as core activities for the training and networking included research on: recirculating aquaculture systems (RAS), artificial feed chains, aquaponics, microalgae, *Litopenaeus vannamei*, native Baltic Sea shrimps, technology optimization, new shrimp tower concept, brackish conditions in freshwater fish RAS, geothermal brine, and *Daphnia* sp. as feed for fish, and provided results crucial for academic and professional trainings, as well as aquaculture business consulting development.

To deliver crucial academic and hands-on-experience training of students and young professionals AquaYouth – "Aquaculture Youth career development" service has been developed, including summer schools on innovative aquaculture technologies such as recirculating aquaculture systems, aquaponics, and integrated systems, carried out at the University of Gdansk and the Klaipeda University. Certified AquaVIP summer schools introduced participants from the region but also from all over the world to background theoretical skills in modern aquaculture biotechnology: main types, biological and technological processes and development trends as well as practical hands-on experience on modern aquaculture technology and innovative blue biotechnology-based approaches. They were based on real ongoing AquaVIP experiments in research facilities, and partner aquaculture companies, and included students' panels and virtual visits to innovative aquaculture facilities, with farms and jobs presentations. AquaYouth service also included study visits in AquaVIP facilities (Klaipeda University and Klaipeda Science and Technology Park) which gave students and researchers a unique opportunity to have an insight into practical aspects of working with large-scale experimental shrimp and fish farms, to share professional knowledge and experience, and find aquaculture themes for further cooperation.

Noticing the necessity to support business performance and development across the modern aquaculture and seafood industry, appropriate trainings and competence building has been offered through the AquaProfi - "Aquaculture Professionals' success support" service. The Master FishFarmer Class (MFFC), a core service activity, with over one hundred forty registered participants focused on the AquaProfi community by inviting professionals and young talents from the South Baltic region. The aim of this exceptional online course, which was based at Rostock University, was dedicated on the one hand for fish farmers aiming at becoming master fish farmers, but also for aquaculture professionals who are looking for further training opportunities. The complete Master FishFarmer Class contained six different topics: RAS technology, larviculture, fish diseases & parasites, algae production, aquafeeds & nutrient requirements & feed additives, aquaculture business & marketing & product development. A seven-months course with each topic lasting approximately for one month, with four online lectures per topic, allowed interested parties to participate in addition to their regular job and gain a certification by passing a final exam and fulfilling course requirements. MFFC gave access to a wide

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spectrum of knowledge and up-to-date-information, provided by our experts, possibility to observe and evaluate familiar processes from a broader perspective, thus developing the professional career, and multiple networking opportunities. The certification was awarded to almost sixty participants from eleven different countries. AquaProfi also included onsite and virtual study visits to innovative aquaculture sites in Germany, Poland and Lithuania. What is more AquaProfi networking activities led to the development of Aquaculture Career Portal, a Linkedin group, launched to share information on courses, exhibitions, study visits, summer schools etc., post jobs and internships offers, look for job and internships possibilities, find partners for your project, business, and more.

AquaTION – "Aquaculture innovation – boosting education and business capacity" is yet a final project deliverable, an e-learning platform which will be developed on the basis of state-of-the-art knowledge and experimental and training activities of the AquaVIP project. To sum up project achievements AquaTION will offer training in skills related to innovative and sustainable aquaculture, crucial for the future employees in the aquaculture sector of the South Baltic area. The aim of the platform is to expand and promote further aquaculture as a blue and green economy sector among employers and employees already active in the labor market, willing to improve their skills, as well as aquaculture students willing to make a career in the aquaculture market in the South Baltic area.

Results

Through its research, educational and promotional activities the AquaVIP project has already resulted in the success stories, and provided a solid base for strengthening aquaculture programs in the universities, provide hands-on-experience for academic communities and create conditions for changes in the labor market. The project has already resulted in two certified aquaculture summer schools, with materials and presentations available on the project website (www.aquavip.edu.pl), where also experiments descriptions, virtual farm visits, videos on good practices, and other educational materials are available. A six-module certified Master FishFarmer Class is another project success, still to be available on AquaTION e-learning platform. Vivid Aquaculture Career Portal offering up-to-date-information and aquaculture job postings guarantees further networking cooperation among partners and stakeholders, strengthened by the development of Aquaculture Competence Centre in Klaipeda, with the plan to extend its activities on the whole of the South Baltic region to bring benefits to aquaculture and related fields students, future employees willing to make a career in the aquaculture market in the South Baltic area, employees and entrepreneurs of micro or SMEs willing to improve their skills, farmers associations, NGOs, authorities interested in improving their skills and sustainable aquaculture development, businesses in the region and society in general.

MINERAL AND VITAMIN NUTRITION IN GILTHEAD SEABREAM JUVENILES (*Sparus aurata*)

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Introduction

Requirements for the major nutrients have been described for several European Union (EU) cultured species including salmonids and carps, however, information related to minerals and vitamins is still scarce for Gilthead seabream (*Sparus aurata*) despite being a major marine species cultured in the EU. Recent studies have described the effects of increasing dietary levels of selenium (Se) (Dominguez et al., 2019a), manganese (Mn) (Dominguez et al., 2020a), copper (Cu) (Dominguez et al., 2019b), zinc (Zn) (Tseng et al., *in prep*), and their sources (Dominguez et al., 2017, 2019c; Mechlaoui et al., 2019; Tseng et al., 2021); as well as vitamin A (VA) (Dominguez et al., *in prep*), vitamin D (VD) (Dominguez et al., 2021, Sivagurunathan et al., *submitted*), vitamin K (VK) (Dominguez et al., 2022, Sivagurunathan et al., *in prep*), and B-vitamins (Dominguez et al., *in prep*)) in gilthead seabream. The present abstract aims to describe the available knowledge on mineral and vitamin nutrition for gilthead seabream juveniles.

Materials and methods

A total of 8 trials were conducted in gilthead seabream juveniles with the aim of understanding the optimum dietary levels for Se, Mn, Cu, Zn, VA, VD, VK and vitamins B1, B9 and B12; an additional 4 experiments were carried out to understand the effects of several mineral sources. For the trials aimed at studying the optimum levels, a basal feed was supplemented with at least 5 different levels for each nutrient, and each feed was tested in triplicates, to give a total of 15 tanks per trial. For the experiments with the difference mineral sources, the same level of the mineral was tested using different sources. Fish were raised by natural spawning at the Aquaculture Research Group from the University of Las Palmas de Gran Canaria ECOAQUA institute, and for each trial the fish came from the same broodstock, so as to reduce the genetic variability. Samples for proximal and vitamin/mineral composition, gene expression, histology and other analyses were taken at the start and end of the trials.

Results and Discussion

Se supplementation up to 0.94 mg/kg promote growth of gilthead seabream juveniles (figure 1). On the contrary, dietary levels of 1.70 mg Se/kg were found to be excessive and caused growth reduction, increased catalase expression and hydropic degeneration in the liver. The Mn content present in the basal diet (19 mg/kg) covered the requirements in juvenile seabream, although results from oxidative status markers might point out the need to increase levels beyond this point when fish are under pro-oxidant conditions. Dietary Cu levels did not affect productive parameters, suggesting that no supplementation was needed. On the contrary elevation of dietary Cu levels up to 11.0-32.0 mg/kg reduced growth and increased the markers of hepatic damage denoting toxic effects of Cu (figure 2). Seabream fed with dietary Zn levels at 89 mg/kg of plant-based diet increased the growth. VA levels tested were several times above those described for other species, however, no effects of toxicity were observed. Increasing the VD level caused an increase in the prevalence of histological alterations in the cardiac muscle. On the other hand, VK supplementation affected growth, *grp* expression, and the reduced prevalence of skeletal disorders suggested that dietary VK levels for gilthead seabream fingerlings should be above 12 mg/kg. Moreover, in an interaction study between VK and VD showed, 0.04mg VD/kg and 12 mg VK/kg in diet reduced total anomalies by upregulating the *bmp2* and *pthr1* expression in vertebral column. Dietary B-vitamins showed no relation to productive parameters, nor were there external signs of vitamin deficiency or mortalities. After the trial, the level of vitamins in whole body had reduced in 50% for B1, 12% for B9 and 18% for B12 on fish fed the diet without supplementation when compared to the initial samples, whereas an increase in the deposition of the vitamins was observed as the dietary levels increased. The dietary increase in B9 and, particularly, B12 reduced the percentage of erythrocytes with irregular nucleus in blood. These results suggest the feeds for seabream should be supplemented with B-vitamins.

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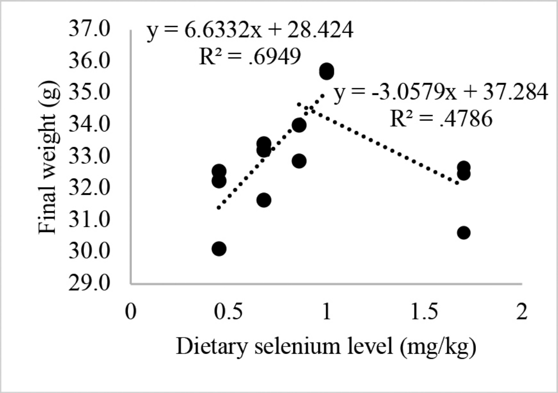

Figure 1. Relationship between dietary selenium level and final weight for gilthead sea bream

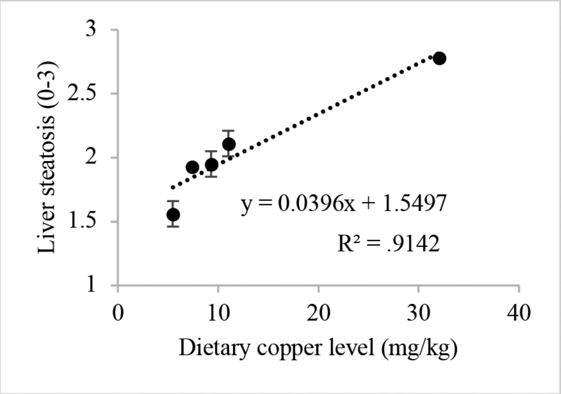

Figure 2. Effect of dietary copper on liver steatosis

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THE PROBIOTIC *Shewanella putrefaciens* PDP11 TARGET VIRULENCE FACTORS BY MODULATING QUORUM SENSING INHIBITION

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Introduction

Bacteria communicate with each other by producing signal molecules and regulating the production of virulence factors that have importance in pathogenicity. Quorum sensing (QS) is a bacterial communication mechanism based on the perception of population density and secretion of determining signal molecules called autoinducers (AI) such as the case of Acylhomoserine lactones (AHLs) (Borges et al. 2019). AHLs-mediated QS processes seem to be common in the marine environment and among marine pathogenic bacteria, which pathogenesis could be mitigated by probiotics (Kuebutornye et al. 2020), among others. Probiotics are defined as live microbial cells that confer health benefits to the host and some of their mechanisms include the production of antagonistic compounds that are inhibitory toward pathogens (Zhou et al. 2018). In this sense, Shewanella putrefaciens Pdp11, a strain described as a probiotic for use in aquaculture, has been analysed to mediate QS processes by quorum-quenching assays using synthetic AHLs.

Material and methods

The detection of AHLs was done as previously described (Torres et al. 2016). *S. putrefaciens* Pdp11 was cultured in tryptone soy agar plates supplemented with NaCl (1.5%) (TSAs) at 23ºC for 24h. Then, the probiotic Pdp11 was grown in 2 mL of tryptone soja broth supplemented with NaCl (1.5%) (TSBs) at 23ºC for 24h under agitation (120 rpm). After 24h, a stock solution of N-butyryl- DL-homoserine lactone (C4-HSL, Sigma-Aldrich), N-hexanoyl- DL-homoserine lactone (C6-HSL, Sigma-Aldrich), N-octanoyl- DL-homoserine lactone (C8-HSL, Sigma-Aldrich), N-decanoyl- DL-homoserine lactone (C10-HSL, Sigma-Aldrich), N-dodecanoyl- DL-homoserine lactone (C12-HSL, Sigma-Aldrich), N-(3-Oxodecanoyl)-L-homoserine lactone (3-oxo-C8-HSL, Sigma-Aldrich), N-(3-decanoyl)-L-homoserine lactone (3-oxo10-C-HSL, Sigma-Aldrich) and N-(3-Oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL, Sigma-Aldrich) were added to achieve a final concentration of 2μg/mL (10μM), and incubated for further 24h. At the same time, an overnight culture of the biosensor *Chromobacterium violaceum* CV026 and VIR07, maintained on Luria-Bertani (LB) plates supplemented with 50μg/mL of kanamycin, and *A. tumefaciens* NTL4 (pZLR4), maintained on LB plates supplemented with 50μg/mL of gentamycin, was diluted to 1:10 in 5 mL of LB soft 0.7% (w/v) agar and poured respectively onto LB agar plates for CV026 and VIR07, and LB medium supplemented with X-gal for NTL4. Then, 6-mm-diameter wells were hollowed in the medium with the back of a sterile Pasteur pipette and 100 µL-aliquots of each Pdp11 culture were loaded in the wells. Simultaneously, 2mL of TSBs were incubated containing AHLs as positive control. The plates were incubated at 28ºC for 24h to check for the presence or absence of a coloured halo around the wells.

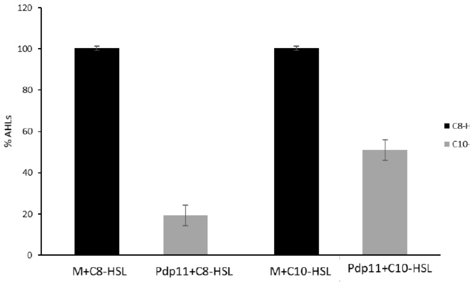

Figure 1. Percentage of synthetic C8-HSL and C10-HSL *N*-acyl homoserine lactones (AHL) measured in the cell-free culture media by degradation of the strain *S. putrefaciens* Pdp11 after 24h. Cell-free medium (M) added HSLs present 100% activity and was used as negative control. Initial AHL concentration was 10 mM. Error bars represent one standard deviation.

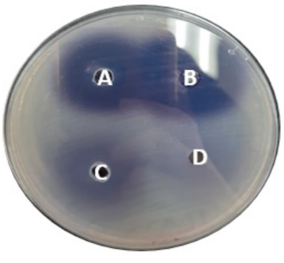

Figure 2. Recovery of synthetic C8-HSL after acidification visualized on agar plate assay by means of the biosensor strain *Chromobacterium violaceum* (CV026). Cell-free medium added with synthetic C8-HSL AB), synthetic C8-HSL extracted by pH7 C) and pH2 D). Scale bar, 1cm.

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To determine whether the QQ activity was related, C8-HSL was added as above in overnight cultures of the degrading strain Pdp11, which was incubated for a further 24h at 23ºC under shaking (120 rpm). The mixtures were then centrifuged at 10000g for 2min, one supernatants were extracted with equal volumes of acetonitrile (1:1) and another supernatants were acidified with HCl 1N to pH 2 and incubated for 24h at 23ºC on shaking. Finally, the remaining AHLs were suspended in methanol 70% (v/v) and detected by well diffusion agar-plate assays using sterile discs and the corresponding biosensor as above. At the same time, to confirm AHL degradation, the final concentration of it in the culture media was evaluated using HPLC-MS.

Results and discussion

Figure 1 shows the degradative activity of *S. putrefaciens* Pdp11 of the synthetic C8- and C10-HSL after 24h. The enzymatic activity is estimated at around 80% and 30% for C8- and C10-HSL, respectively, while the rest of AHLs tested were not degraded by the Pdp11 strain. It would be an interesting feature of the probiotic Pdp11 strain since these AHLs are related to facilitating microbial adhesion by promoting biofilm formation among other virulence factors related to pathogens (Fan et al. 2019).

On the other hand, a distinctive feature of AHL inactivated by lactonase is that it can be reactivated by acid treatment (Shaheer et al. 2021). In this way, little C8-AHL was recovered when it is extracted to pH2 (Figure 2), which indicates the enzyme activity is not derived from the hydrolysis of the lactone ring derived from the action of lactonases (Yates et al., 2002), suggesting the enzyme activity in Pdp11 could be an AHL-acylase. The potential QQ activity of Pdp11 was unknown so, these preliminary studies led to a further as another promising probiotic QQ tool for aquaculture.

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ENVIRONMENTAL SALINITY REGULATES BARRIER FUNCTION IN RAINBOW TROUT SKIN

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Introduction

The skin is one of the primary barriers between the fish and the external environment. As such, the skin is a vital organ in osmoregulation, where it acts as a barrier against ion diffusion. Additionally, the health of the skin is vital in aquaculture settings, where fish may be exposed to an array of pathogens and other contaminants. However, relatively little is known about how the skin functions in relation to environmental variation.

The aim of this study was to measure barrier function and active transport in the trunk skin of rainbow trout using the Ussing chamber technique. The effects of epithelial wounding, skin region, and salinity on skin function were tested. In addition, immunohistochemistry was used to test for the presence of certain ion transporters including V-ATPase, NKA, and NKCC.

Method

The contribution of the different skin layers (epidermis and dermis) to barrier function and active transport was assessed in fully intact skin (dermis, epidermis, and scales present) and wounded skin (only dermis present). Regional differences in skin function were also assessed, as well as the response of the skin to environmental variation in the form of salinity change. To explore barrier function and active transport in long-term acclimated fish, skin sampled from freshwater (FW) acclimated fish was exposed to FW (FW-FW), while skin from seawater (SW) acclimated fish was exposed to SW (SW-SW). To explore the effect of acute salinity change on barrier function and active transport, skin from FW acclimated fish was exposed to SW (FW-SW), while skin from SW acclimated fish was exposed to FW (SW-FW). Each salinity exposure took place on the apical/epidermis side. Finally, the effect of pharmacological inhibition on potential sites of active transport in the skin was assessed.

To assess skin barrier function and active transport, the Ussing chamber technique was used. This method involved the measurement of several electrophysiological parameters from excised skin sections. These measurements included transepithelial resistance (TER), transepithelial potential (TEP), and short-circuit current (SCC). The TER mainly reflects the paracellular shunt resistance created across the tight junctions (TJs) between the epithelial cells, while the TEP reflects the net ion distribution across the epithelium as a result of the paracellular and transcellular transfer of ions. Finally, SCC is a measure of the net active ion transport across the epithelium. Skin sections were gently removed from each fish using a scalpel and locking forceps, according to Figure 1. The basal side of the skin was bathed in Ringer's solution and the apical side was bathed in either freshwater or seawater.

Results

The results of our study indicate that salinity is the primary regulating factor on both barrier function and transport in the trunk skin. At isosmotic conditions, the skin has a very small transepithelial potential, indicating little active transport. However, we found that skin permeability decreased drastically following exposure to freshwater and increased following exposure to seawater, with these changes occurring rapidly. Changes in epithelial permeability were accompanied by salinity-dependent changes in transepithelial potential (TEP) and short circuit current (SCC; Figure 2). We propose that barrier function in rainbow trout trunk skin is regulated by TJs that rapidly respond to changes in salinity. These changes are likely due to the varying expression of claudins in both freshwater and seawater. To assess the skin for active transport, we exposed to the skin to the V-ATPase inhibitor N-ethylmaleimide. This resulted in a gradual increase in epithelial permeability and changes in TEP, in both freshwater and seawater, suggesting an opening of the TJs. The presence of V-ATPase was confirmed using immunohistochemistry. However, there was no evidence that NKA or NKCC were present in the skin.

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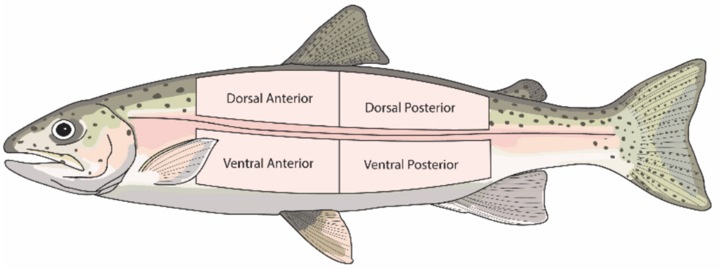

Figure 1. Diagram showing the skin regions that were sampled to assess regional differences in barrier function and active transport. The same skin regions were sampled from both the right and left side of each fish.

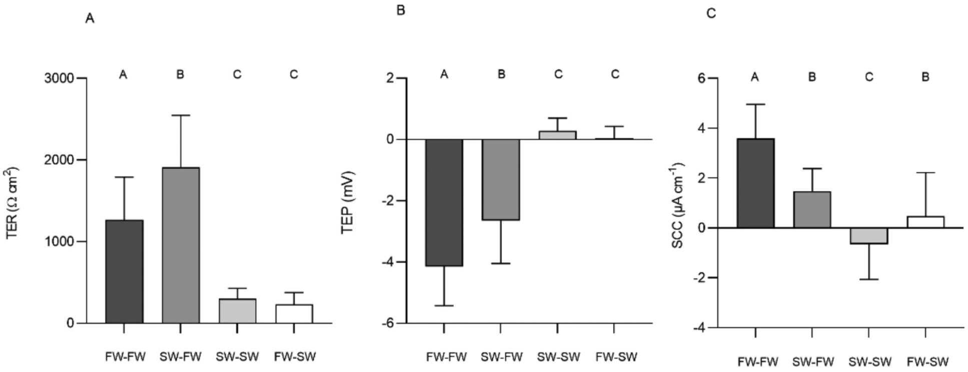

Figure 2. Barcharts showing the effect of salinity on (A) transepithelial resistance (TER), (B) transepithelial potential (TEP), and (C) short-circuit current (SCC). Bars represent means and whiskers represent SD. Groups not sharing a letter differ significantly based on the results of Welch's ANOVAs and post-hoc Games-Howell tests.

Discussion

In this study, we assessed the contribution of the different skin layers to barrier function, determined the effect of salinity, and tested for possible active transport mechanisms. The epidermis was found to be the diffusion barrier in the skin, with the dermis playing a negligible role. Epidermal barrier function was mainly determined by environmental salinity, with FW exposure resulting in a strong reduction of epithelial permeability. While the rapid salinity-dependent changes in skin function are likely related to acute changes in TJ structure and function, the exact mechanisms behind the acute changes in epithelial permeability remain unclear. However, the salinity dependent differences in barrier function observed in the current study are consistent with previous studies that have showed varying expression of TJ proteins in the skin of fish. In relation to active transport, immunostaining and selective inhibition suggest that one active transport component is an apical V-ATPase. However, further research is required to determine the exact role of this transporter in the context of the trunk skin.

RE-THINK PHOSPHORUS: HOW SALMON BONE REACTS TO LOW AND HIGH PHOSPHORUS DIETS

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Introduction

The main minerals of bone are calcium and phosphorus. While teleost can obtain calcium in sufficient amounts from water they depend on dietary P supply. Dietary P deficiency is considered a risk factor for the development of vertebral deformities in farmed Atlantic salmon (*Salmo salar*). In commercial diet formulations dietary P requirements are met by addition of inorganic P compounds such as mono-ammonium phosphate (MAP) which are highly digestible (up to 90%) (Morales et al., 2018). Recent studies on early seawater stages of Atlantic salmon show that low-P diets result in the development of non-mineralised bone matrix that retains the capacity to mineralise without causing vertebral deformities (Witten et al., 2016; 2019). High P diets, on the other hand, may increase P excretion associated with waste of P resources and environmental pollution. We here present results from two studies that show the effect of low and high dietary P on freshwater stages of Atlantic salmon and the long-term effects of dietary P history at harvest. Study 1 analysed the effects of dietary P as a single factor for skeletal health. Atlantic salmon parr were fed low and high P diets and growth performance, plasma P content, and vertebral deformities were analysed. Study 2 followed individually tagged animals with low and high dietary P history from study 1 up to harvest. Progression and regression of deformities related to the animals' dietary freshwater history were analysed.

Materials and Methods

Study 1: Freshwater parr stages of Atlantic salmon (start weight 13.5 g) were fed MAP supplemented diets with a low (0.35%), regular (0.56%), or high (0.93%) estimated available P for 11 weeks (week 0 - 12). Animals were subjected to different analytical methods such as radiography, whole mount Alizarin red S staining, histology, plasma analysis, and bone mineral content analysis.

Study 2: The remaining animals from the study 1 (45 animals per diet group) were tagged and fed a commercial diet with regular P content for 66 weeks in seawater up to harvest (4.5 kg). Deformity development was monitored in individual animals by repeated radiology (at week 15, 47, 84) up to harvest. Additional analysis was done by Alizarin red S whole mount staining, histology, and bone mineral analysis.

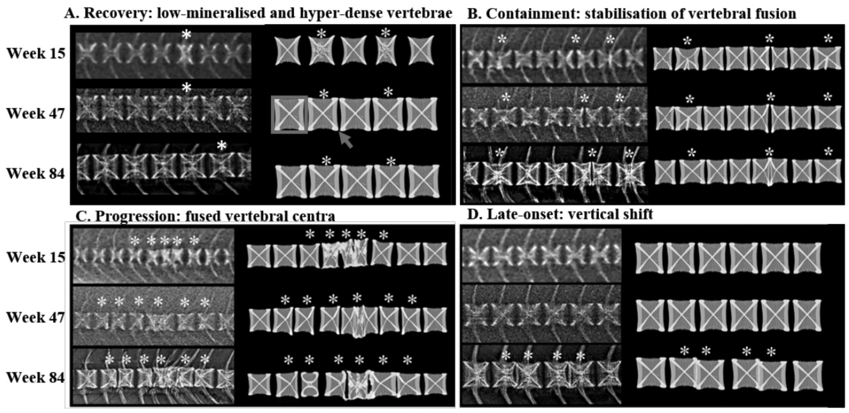

Figure1. **Development of vertebral deformities.** X-ray images and cartoons of individual Atlantic salmon x-rayed at week 15 (freshwater), 47, and 84 (seawater). Each block represents one animal. Deformities with the potential to recover (A) affected bone trabeculae of the vertebral centra (rectangle) but not the intervertebral spaces (arrow). Deformities which affected both structures were either contained (B) or progressed (C). Late-onset deformities developed in *de-novo* in seawater (D).

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Results and Discussion

Study 1 (freshwater): The prevalence of deformities in Atlantic salmon (week 12) was low and there was no effect of low or high P diet on the development of deformities. Animals fed low-P diet showed low-mineralised vertebrae characterised by extended areas of non-mineralised bone. Plasma P levels showed a 3-fold reduction in low-P fed animals. While bone formation continued, bone mineralisation was discontinued. In contrast, regular-P and high-P diet animals had fully mineralised bone. The high-P diet had no further effect on bone mineralisation. Interestingly, the growth of animals fed different levels of dietary P was comparable, no growth retardation was observed in low-P diet animals (Drábiková et al., 2021).

Study 2: Vertebral body deformities either affected bone trabeculae of the vertebral centra (rectangle in Fig. A) or the intervertebral spaces (arrow in Fig. 1A). Deformities of the vertebral centra with intact intervertebral spaces (low-mineralised, hyper-dense, compressed, and vertically shifted vertebrae) (Fig. 1A), had the capacity to recover in seawater (week 47). The prevalence of all deformities was low, except low-mineralised vertebrae and hyper-dense vertebrae that were frequent in low-P diet animals. In seawater low-mineralised vertebrae were completely mineralised and hyper-dense vertebrae recovered. Deformities which affected both the vertebral centra and the intervertebral joints, equally prevalent in all diet groups, were either contained (stabilised vertebral fusion) (Fig. 1B) or progressed over time (fused vertebral centra) (Fig. 1C). Deformities could also develop late in the seawater (week 47-84) (late-onset vertical shift and compression) (Fig. 1D). At harvest (week 84), the number of progressive deformities was low and independent of dietary P history.

In current studies the dietary P in freshwater has an immediate effect on bone mineralisation but no effect on the development of bone deformities and growth. There was also no long-term effect on the incidence of deformities at harvest. This study shows that under favourable conditions specific deformities can recover in seawater. Moreover, a high-P diet freshwater history had no effect on growth or development of deformities.

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EFFECT OF INSECT MEAL AND INSECT OIL ON PERFORMANCE, DIGESTIBILITY AND RESPONSE TO CHALLENGE TEST WITH *Vibrio parahaemolyticus* IN JUVENILE WHITE SHRIMP (*Litopenaeus vannamei*)

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Introduction

Insect-based products are gaining in popularity as a good sustainable protein and oil alternative for animal feeds. Beyond fishmeal replacement, insect meal and oil present potential functional benefits to the farm-raised animals. In the present paper insect meal and oil, from Black Soldier Fly (*Hermetia illucens*) larvae were tested at different inclusion rates in shrimp (*Litopenaeus vannamei*) diets.

Methods and Materials

The trial aimed at monitoring zootechnical performances including growth, survival and feed conversion ratio (FCR), and immune responses in laboratory conditions of PL12 individuals for a 45 days period. A seven day challenge test with *Vibrio parahaemolyticus* was also conducted on those juveniles where mortality was recorded and hepatopancreas histology compared between the control and test diet.

Five experimental groups, each with four replicates, were studied as follows: 2% Insect meal, 5% Insect meal, 10% Insect meal, 2% Insect meal + 2% Insect oil, and a control (without the inclusion of insect meal or insect oil).

Results

After 45 days of feeding, shrimp fed 2% Insect meal + 2% insect oil, and 10% Insect meal had a significantly higher average survival rate than other groups. At the same time, shrimp provided 2% Insect meal +2% Insect oil, 5% Insect meal, and 10% Insect meal, had a significantly higher average body weight and lower FCR than other groups. Regarding the immunological study, shrimp from all groups that provided insect meal had significantly higher immune responses (the total hemocyte count, phagocytosis activity, phenoloxidase activity, superoxide dismutase activity, and bactericidal activity) than the control group. Shrimp from all groups that provided insect meal had fewer *Vibrio spp* in the hepatopancreas and intestine of shrimp than other groups.

Shrimp provided with 2% Insect meal +2% Insect oil had the best apparent digestibility of nutrients, including a percent dry matter digestibility, percent organic matter digestibility, percent protein digestibility, percent lipid digestibility, and percent nitrogen-free extract.

In the challenge test, all insect-based treatment showed a 26% better survival than the positive control (no insect ingredient). Furthermore, a noticeable reduction in the sloughing signs of the hepatopancreas was observed in the tested diets.

Conclusion

These results indicate health and performance benefits of insect meal and oil in shrimp diets in controlled conditions.

HYDROLYZED YEAST *Kluyveromyces fragilis* AS A FEED MATERIAL TO IMPROVE GROWTH PERFORMANCE AND IMMUNE FUNCTIONS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Functional feed materials derived from yeast are a highly valued ingredient in a wide range of animal diets. While most of these products for aquaculture are based on *Saccharomyces cerevisiae*, the use of *Kluyveromyces fragilis* (also named *K. marxianus*) has hardly been investigated. In previous studies, *K. fragilis* showed a higher nutritional value compared to *S. cerevisiae*, as evidenced by a higher proportion of fish meal replacement in Atlantic salmon diets (Øverland et al. 2013). To further improve the digestibility and efficiency of functional components such as mannans, ß-1,3-1,6-glucans and nucleotides, the product TechnoYeast – hydrolyzed *K. fragilis* (KF) – was created. The purpose of this trial was to investigate the effects of different dosages of KF on growth performance and immune parameters of rainbow trout (*Oncorhynchus mykiss*).

Material and methods

2400 fingerlings (avg. weight 16.3g) were randomly stocked into 4 concrete outdoor raceway ponds (600 each). The raceways were separated by metal mesh to get triplicates of 200 fish for each group. After two weeks of acclimatization, the 90 days feeding period was started. The control group (CON) was fed a commercial trout feed with 44% crude protein, 15% fat, 3% crude fiber and 9% ash. For the treatment groups KF (TechnoYeast, Biochem GmbH, Lohne, Germany) was added to the commercial feed via gelatin. The groups were defined by the inclusion level of KF as KF 0.5%, KF 1.0% and KF 2.0%. Feeding was performed 4 times a day according to the standard table of the feed supplier and based on 1.5% to 2% of the average body weight considering the water temperature. Due to seasonal changes, the water temperature was 10 to 13°C for the first 30 days and 4 to 8°C for the last 60 days. The pH was around 7 and oxygen level was >9 mg/l. At the end of the feeding period, 10 fish from each replicate were randomly selected for biomass measurements and to take blood samples. Blood from each replicate was pooled, separated with heparin, centrifuged at 3000 rpm, and sent for laboratory analysis. Standard statistical tests (ANOVA) were used to calculate significance values.

Table 1: The table indicates mean performance results and mean blood analysis values at the end of the 90 days feeding period of rainbow trout fingerlings.

	CON	**KF 0.5%**	**KF 1.0%**	**KF 2.0%**
Performance parameters				
Initial weight [g]	$17.2^{a} \pm 0.5$	$17.8^{a} \pm 0.7$	$17.1^{a} \pm 1.2$	$12.9^{b} \pm 2.5$
Final weight [g]	$81.4^{a} \pm 2.0$	$91.2^{b} \pm 4.6$	$94.6^{b} \pm 6.5$	$102.9^{c} \pm 5.3$
WG [g]	$64.2^{a} \pm 1.5$	$73.5^{b} \pm 5.0$	$77.5^{b} \pm 7.0$	$90.0^{c} \pm 7.3$
SGR [%BW/day]	$1.73^{a} \pm 0.01$	$1.82^{a} \pm 0.08$	$1.90^{a} \pm 0.12$	$2.31^{b} \pm 0.24$
FCR	$0.79^{a} \pm 0.01$	$0.73^{a} \pm 0.04$	$0.69^{a} \pm 0.05$	$0.61^{a} \pm 0.05$
Survival [%]	99.98^{a}	99.33^{a}	99.67^{a}	99.98^{a}
Immunity parameters				
WBC [cells/µm^3]	$13.3^{a} \pm 1.25$	$12.2^{ab} \pm 1.11$	$9.7^{bc} \pm 0.76$	$7.5^{c} \pm 0.60$
ALT [u/l]	$22.0^{a} \pm 1.00$	$16.3^{b} \pm 2.30$	$18.6^{ab} \pm 2.08$	$14.7^{b} \pm 1.15$
AST [u/dl]	$21.6^{a} \pm 0.26$	$17.6^{b} \pm 0.38$	$18.5^{b} \pm 0.61$	$13.6^{c} \pm 1.03$
LDH [u/cl]	$17.6^{a} \pm 0.79$	$10.7^{b} \pm 0.51$	$11.9^{b} \pm 0.77$	$8.6^{c} \pm 0.65$

WG: weight gain; SGR: specific growth rate; BW: body weight; FCR: feed conversion rate; WBC: white blood cells; ALT: alanine transaminase; AST: aspartate transaminase; LDH: lactate dehydrogenase; ^{abc} means with different superscripts within a row differ significantly ($P < 0.05$)

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Results and discussion

At the beginning of the trial fish of the KF 2.0% group had a slightly lower initial weight compared to other groups. However, these fish achieved the highest final weight of all treatments (Tab. 1). This compensatory growth could be related to higher feed efficiency, which is evident in all KF groups through improved FCR and increased SGR. These results are consistent with previous trials with KF in terrestrial animals, e.g., in pigs (Keimer et al., 2018). The performance data in Table 1 generally shows a scalable and significant increase in performance with increasing dietary inclusion levels of KF. Analysis of the blood samples revealed that the white blood cell (WBC) count in the fish was negatively correlated with increasing dietary inclusion of KF (Tab. 1). In previous studies, high WBC counts were associated with the influence of various types of stress (Peters & Schwarzer 1985). Consequently, all KF-groups appeared to be less affected by stressors such as pathogens or handling than the control group, resulting in better growth performance.

Further analysis of the liver enzymes lactate dehydrogenase (LDH), aspartate transami-nase (AST) and alanine transaminase (ALT) displayed the positive effects of KF on the overall health status of the fish (Tab. 1). AST and ALT are common indicators of liver condition. Elevated levels of these enzymes are often a result of intoxication (Bury et al. 1997). Besides, increased LDH levels usually occur in association with tissue damage. Thus, studies with other freshwater fish species showed an increase in LDH when exposed to sewage (Parveen et al. 2017). Herein bad water quality caused cellular hypoxia, creating anoxic condition and cell damage. A similar event occurred during the feeding period of the present study when heavy rain flushed the flow-through system with turbid water in week 6. Interestingly, all groups fed with KF had lower concentrations of the analyzed liver enzymes when compared to the control, which could be a sign for a better protection against such challenges.

In conclusion, KF positively affected immunity and health parameters of rainbow trout fingerlings. All dietary inclusion levels tested significantly improved growth performance, with the strongest effects observed at a 2.0% dosage.

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EVALUATION OF NUTRIENT AND LIGHT REQUIREMENTS OF TROPICAL MACRO-ALGAE IN VIEW OF INTEGRATED SHRIMP CULTURE WITH SHRIMP IN RAS

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Driven by product diversification and circularity of nutrients, also land-based production systems are exploring the potential of integrated aquaculture. The co-cultured species are used as biofilters, and each level has its own independent commercial value, providing both economic and environmental sustainability.

In this study, the nutrient uptake of *Caulerpa lentillifera* was evaluated together with its light preferences in view of integrated cultivation with *P. vannamei*.

The growth performance of the macro-algae and its uptake of NO_3-N, PO_4^{3-} and NH_3-N at three different concentrations of organic fertilizer (233μlL^{-1}, 466 μlL^{-1}, 932 μlL^{-1}) was evaluated for 28 days. Growth performance was best at 233 μlL^{-1} and lowest at 932 μlL^{-1} of organic fertilizer. A reduction in NO_3-N and PO_4^{3-} of 77,4% and 3%, respectively, was registered together with an increase of NH3-N of 19,2%, indicating a preference of Caulerpa for nitrate over ammonia under experimental culture conditions. In a subsequent experiment, the effect of light quality (spectra) on the growth of *Caulerpa lentillifera* was determined. The results indicated a preference for blue light, whereas the lowest growth performance was under warm white light conditions. The third and final experiment revealed a better growth performance under LED light over fluorescent light. In future experiments, the present results will serve as basis for the integrated culture with shrimp and indicate a nitrate reduction of the culture water in comparison to monoculture. The first experiment will be repeated using more accurate techniques for nutrient analysis to verify the rise in NH_3-N.

This study was realized thanks to co-funding from the Europees Fonds voor maritieme zaken en visserij (EFMZV) and the Vlaamse Visserij- en Aquacultuursector (FIVA).

Europees Fonds voor
Maritieme Zaken en Visserij

Europese Unie

AQUACULTURE FEEDS AND OFF-ODOURS COMPOUNDS IN RECIRCULATING AQUACULTURE SYSTEMS: CORRELATIONS, PATHWAYS AND MITIGATION THROUGH ALTERNATIVE INGREDIENTS

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Introduction

The presence of off-odour compounds in fish, such as geosmin and 2-methylisoborneol (2-MIB, Abd El-Hack et al., 2022), or less commonly reported skatole, eugenol, or indole (Mahmoud et al., 2018), can drastically reduce its palatability, leading to consumer rejection and lower market demand of recirculating aquaculture systems (RAS) products. A common off-flavour mitigation strategy is to purge the fish in flow-through systems prior to harvesting while ceasing feeding. However, this procedure results in the loss of fat content and precious market weight, as well as in a far greater consumption of water. Other technological strategies utilise intense ozone and ultraviolet (UV) light, which further increase costs in RAS farms. In the last 30 years, scientific effort has been directed towards understanding and mitigating the presence of bacteria lineages believed to be responsible for the production of geosmin and 2-MIB for quorum sensing. Previously, these compounds were believed to be only absorbed by aquaculture fish from the water, yet recent work has demonstrated the presence of these compounds and other dominant off-odour taints in aquaculture feeds, indicating their potential absorption through ingestion (Podduturi et al, 2017; Mahmoud et al., 2018).

Methods

This presentation outlines a conceptual workflow to address the issues of off-odour in RAS fish in relation to feed. The present work will apply sensory evaluations combined with analytical methods (e.g., solvent assisted flavour evaporation (SAFE) technique, gas chromatography-mass spectrometry/olfactometry (GC-MS/O), aroma extraction dilution analysis (AEDA), proton transfer reaction-time of flight-mass spectrometry (PTR-TOF-MS), stable isotope dilution analysis (SIDA)) to explore correlations between dominant off-odour compounds in common aquaculture feeds and their presence in RAS water and several RAS fish species. The study will examine the pathways of production, absorption and bioaccumulation of off-odour compounds. After determining links between feed ingredients and off-odour formation, new fish feeds will be formulated and trialled. The formulations will aim to reduce off-odour presence via feeding by employing only plant/algae-based ingredients exhibiting no off-odour themselves (Garcia-Vaquero & Hayes, 2016) and then testing various chemically mediated mitigation strategies. Overall, the project aims to develop and maintain a cost-effective feed while improving digestibility and nutritional/welfare requirements of the fish.

Expected Results

The expected outcomes of this project are an understanding of the relationship between aquaculture feed ingredients and off-odour in fish, as well as the evaluation of which feed parameters impact microbial and water quality and fish health. Further, the use of plant/algae ingredients as an alternative to fishmeal will be assessed, with related strategies, to prevent the formation of off-odours in RAS fish.

Points of Discussion

Aquaculture feeds represent the focal point at which the aquaculture industry and marine environmental conservation meet. Currently, the research and development of alternative feed ingredients receives unprecedented attention from private and public investors (Naylor et al, 2021). This is motivated by a set of fundamental strategic priorities: producing larger amounts of feeds that employ cheaper and more readily available ingredients than fishmeal/fish oil; shifting away from the dependency of stagnant baitfish fishery landings; and completely lifting the fishery pressure on wild stocks to let world oceans recover from overfishing. Further, aquaculture feeds today represent the highest capital expenditure for aquaculture farmers, amounting up to 34% (Liu et al., 2016) of the total final product in RAS farms. Finally, the design of RAS depends on the feed loads, protein content and conversion ratios, which increase the dependency of RAS farmers on digestible and highly nutritional feeds. Consequently, a great research effort is required to improve aquaculture feed formulation. This

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work can provide means to reduce off-odours in the feeds by employing alternative feed ingredients, while potentially improving digestibility and promoting a healthy microflora in the animals' gut and biological filters. Such findings could promote high flavour quality product standards and improve cost-effectiveness in RAS production.

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METABOLIC FINGERPRINTING OF RECIRCULATING AQUACULTURE SYSTEMS: A PILOT APPLICATION OF UNTARGETED METABOLOMICS TO DESCRIBE BIOLOGICAL RECTORS

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Introduction

In land-based recirculating aquaculture systems (RAS) the removal of nitrogenous waste is achieved via prokaryotic biodegradation. However, the spatiotemporal metabolic dynamics of microbial communities associated to this process in RAS are poorly understood (Letelier-Gordo *et al.,* 2020). Fast and effective top-down molecular techniques might aid into better understanding these dynamics to suggest improvements in process design and operation (Vinayavekhin & Saghatelian, 2010; Xiao *et al*., 2019).

Methods

This pilot study evaluated the applicability of untargeted metabolomics fingerprinting to describe metabolic trends in RAS bioreactors. We compared two reactor designs, a two-stage moving bed bioreactor (MBBR) and an anaerobic batch fed sludge bioreactor (BFDR), as well as different locations within each of these (i.e., inlets vs outlets).

Results

The untargeted LCMS approach highlighted a clear differentiation in metabolic fingerprints between the two bioreactors. No difference was found within the MBBR (Fig. 1). However, two distinct metabolic fingerprints were recorded within the BFDR, one in the reactor's sludge blanket and one in the supernatant.

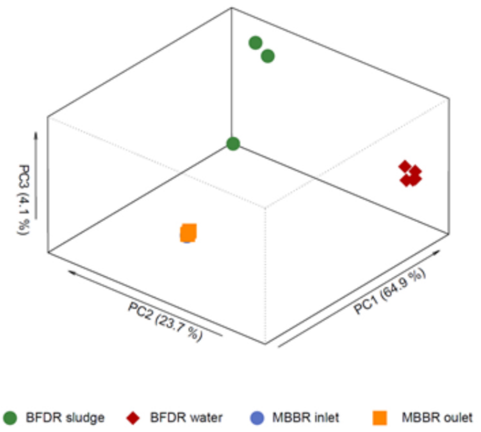

Fig. 1. PCA plot summarising 92.9% of variance in three principal components. Signals of MBBR inlet and outlet are clustered, while BFDR water and sludge are clearly distinct.

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Discussion

Contrarily to the initial hypotheses, MBBR signals clustered. Possible explanation can be identified in a vessel-wide distribution of a homogenous microbial community, influenced by substrate-limiting conditions and short hydraulic retention time (Torresi *et al.,* 2018; Yang *et al*., 2019). Unexpectedly, the BFDR showed a significant diversity in metabolome, inferring on a significant differentiation between location-specific microbial communities. We demonstrated how untargeted metabolomics can reveal metabolic dynamics in a RAS bioreactor and at the same time confirm or deny their initial design principles.

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AQUAGRAPH – USING GRAPH DATABASES TO VISUALIZE AND OPTIMIZE DECISIONS IN AQUACULTURE

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Introduction

The aquaculture industry produces enormous amounts of data every day. Data comes from sensors in the fish cages, cameras, boats, feeding barges, but also from e-mails, notes, and through conversations between the people working on the production sites. Some tools exist for gathering and displaying the produced data, and there are several initiatives for standardizing the data, but there are also challenges with today's solutions related to finding and visualizing the interconnections between the gathered data. When the data quantity is high the interconnections between data become more important. Furthermore, it is difficult for humans to navigate through millions of data points in a table and find these interconnections. A graph database [1] stores relationships and connections between data, as well as the data itself, and is therefore well suited for this particular purpose. The Aquagraph project (RCN 321422) aims to implement aquaculture data in a graph database and develop methods and algorithms that will aid the fish farmers in their daily operations as well as in their long-term planning. The project group consists of the fish farming company Eide Fjordbruk [2], one of Europe's largest ocean research organizations, SINTEF Ocean [3], and leading data scientists from the innovative company and project owner Searis AS [4].

Materials and methods

The project utilizes the Neo4j graph database software to store and structure the data [5]. The Neo4j software features extensive libraries for graph representations and multiple algorithms allowing searches, graph traversal, and clustering, amongst others.

Prior to bringing the data into a graph database the assets that belong to an aquaculture production process, and the relationships between them, need to be given a formal name and a definition in the form of an ontology data model [6]. The data model defines the general concepts in a domain context, e.g., the aquaculture fish farming operations. Once the ontology is in place one can easily add and remove assets from the graph, and the graph database will automatically ensure that the asset has the correct properties and relationships. As such, scaling and updating a graph database is fast and effective, compared to traditional databases. Figure 1 shows how some assets and the life cycle of the fish are described in the ontology, while Figure 2 shows how these are implemented in the graph database and connected to all the proper dependencies, with the correct relationships and properties. Other assets included in the ontology can be listed as sensor data, batches of fish, observations, actors, and events.

The software Clarify [4], developed by Searis, is used in the project to represent time-series data from sensors, but also as a platform for operators to communicate. Clarify serves the graph database with data in form of sensor measurements, time stamps of important events (e.g., delousing operations and feeding times), inputs from the operators, and maybe most importantly: Anomaly detections that, when represented in a graph, will make it simple for the operators to detect if something needs attention.

Results

Initial results from the project includes a complete ontology for aquaculture data structuring. Furthermore, a graph database representation of all aquaculture assets at one of Eide Fjordbruk's fish farms has been implemented in Neo4j. The graph database, and graph visualization, have been applied to new data, but historical data has also been implemented. By doing this it is possible to compare historical productions that e.g., has been very good, to new, ongoing productions, and determine if there are differences between them, and where one should focus the effort e.g., leading up to a delousing operation or when splitting and merging groups of fish.

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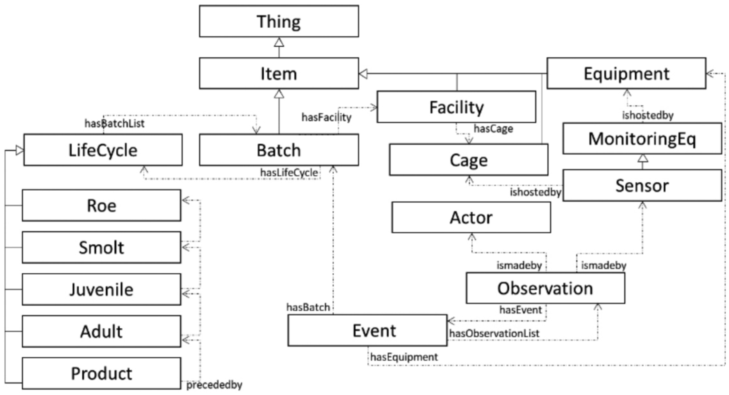

Figure 1: The ontology describing different assets in the production and the fish's life cycle.

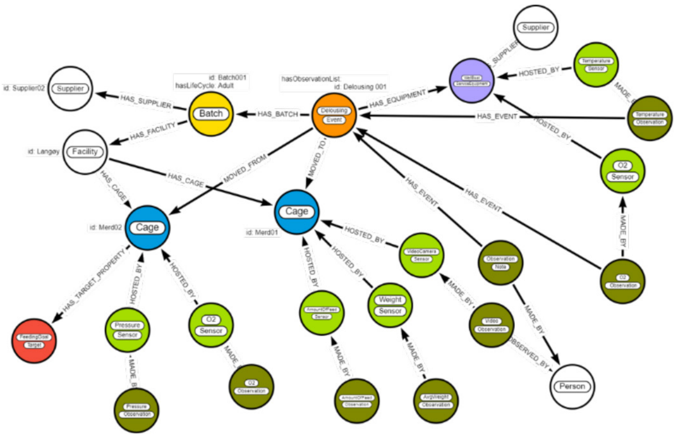

Figure 2: The ontology represented as a graph in Neo4j.

Conclusion and future work

The results from the Aquagraph project, i.e., the new way of visualizing aquaculture data and the optimization algorithms, may enable the fish farmers to learn more about the cause and effect of their actions, the significance of various environmental parameters, and may make it easier to track a group of fish throughout a production cycle, from egg to slaughter. The graph database representation can lead to the discovery of new relationships that were previously underestimated or unknown to the fish farmers. It will help put the important data in front of the right people which may secure a better foundation for decision making. The project lays the foundation for the implementation of new tools on top of the existing Clarify platform. Furthermore, machine learning can be applied to predict outcomes of production, and future relationships which were previously unknown. The project group believes that augmenting the framework with machine learning capabilities will be beneficial to the project since this opens for the use of not only machine learning plus data, which is good, but machine learning, plus data, plus context of data, which is better.

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BLOOD AND MILT ANDROGENIC PROFILES OF EUROPEAN EEL (*Anguilla Anguilla*) AND CORRELATION WITH SEMINAL MORPHO-FUNCTIONAL CHARACTERISTICS

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Introduction

In the last decades, the population of European eel (*Anguilla anguilla*) has suffered a strong decline due to overfishing, habitat reduction and pollution, and the constant growth of the market's demand [1]. As a matter of fact, the European eel population has declined by 98% since 1980 and is currently listed as a "critically endangered" species by the International Union for Conservation of Nature (IUCN) Red List [2]. In captivity, when the reproductive migration is not allowed, eels do not mature spontaneously, and long-term hormonal stimulation is necessary to promote correct gametogenesis. As a matter of fact, male specimens start producing high quality ejaculates only after 13-15 weeks of treatment with human chorionic gonadotropin (hCG) [3,4]. In fish, the principal androgen related to spermatogenesis and testis development is 11-keto-testosterone (11-keto) alongside its precursor, testosterone (T). The peak of androgens occurs some week post hormonal treatment and reaches its maximum when spermatogenesis starts [5]. The aim of the present work was to quantify 11-keto and T in both milts and bloods of male European eels and correlate them with semen morpho-functional parameters such as spermatozoa concentration, viability and objective motility.

Material and Methods

Twenty-four (N=24) male European eels were sampled at the end of the above-mentioned hormonal treatment with hCG. Upon anesthesia, 500 µL of blood and 2 mL of milt were samples from each specimen. An aliquot of milt was immediately diluted 1:10 in P1 medium added with BSA 2% w/v (4°C; ph=8.2) for the semen morph-functional evaluations while blood and the remaining milts were centrifuged (3000g x10 min). The resulting plasmatic components of both matrices were stocked at -20°C waiting for hormonal extraction and quantification. Sperm concentrations were evaluated using a haemocytometer, viability by mean of Eosin-Nigrosin staining. After spermatozoa activation with Artificial Sea water (3.7 %) at 4°C, motility was evaluated by CASA analysis.

11-keto and T were extracted in ethyl ether and quantified, respectively, using ELISA and Radio-immunological (RIA) methods. Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA). Descriptive statistic included mean, SD and normal distribution by Shapiro-Wilk test. Spearman's rank correlations were performed to evaluate the correlation between androgens and sperm parameters. The statistical significance for all tests was set at $p < 0.05$ (95% C.I.).

Results

The results showed that it was possible quantify both androgens in all milts (T= 0.24 ± 0.04 ng/mL; 11-keto= 1.09 ± 0.70 ng/mL) and blood (T= 2.02 ± 0.53 ng/mL; 11-keto= 6.89 ± 3.56 ng/mL) samples. As for milts morph-functional parameters we recorded mean spermatic concentration of 1.70×10^9 spz/mL, mean viability of 94.1% and mean total motility of 52.76%. The statistical analysis did not show any correlation for the androgens between blood and seminal plasma. Testosterone and 11-Ketotestosterone resulted correlated in blood ($\rho=0,67$; $p<0,001$) but not in seminal plasma. The results of the morpho-functional evaluations showed a positive correlation between sperm viability and motility ($\varrho=0,46$ $p=0,024$), but no correlation was found between spermatozoa concentration and other parameters. No correlations were highlighted by the analysis between the level of hormones in blood and seminal plasma and the sperm quality evaluation (motility, viability and concentration).

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Discussion and Conclusion

The present study seems to be indicative of a lack of correlation between the androgen profiles of blood and seminal plasma, with the only exception of an evident relation between 11-keto and T within blood. Such study also highlights the need for a thorough evaluation of the morph-functional characteristics of milts, as assessing just one parameter such as spermatic concentration does not seem to be indicative of overall quality. Finally, it is important to mention that the lack of correlations between the analyzed hormones in seminal plasma and milts' quality suggests the need the keep on investigating the European eel's physiology of reproduction and in particular spermatogenesis, as other hormones, not fully investigated to date, may influence sperm concentration, viability and motility.

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VITAMIN C ADMINISTRATION PROMOTES THE PERFORMANCE OF CCB CELLS AND CARP-DERIVED LEUKOCYTE DURING INFECTION WITH KOI HERPESVIRUS

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Introduction

Most freshwater teleosts lack vitamin C (vitC) or ascorbate due to the absence of L-gluconolactone oxidase, the enzyme responsible for ascorbic acid (AA) synthesis [1,2,3]. Deficit species, including carp usually require a dietary intake of vitC. AA plays a fundamental role as growth promoter and immune protective agent in freshwater fish [2,3]. Despite the vitC reputation as a robust antioxidant with anticancer capabilities, its antiviral function is poorly investigated. We examined the immune potential of AA in-vitro in brain cells and leukocytes of common carp *Cyprinus carpio* L. amid koi herpesvirus (KHV) infection as crucial cells for KHV replication and latency and carp fish immunity.

Materials and Methods

To experiment vitC influence on cell performance, naïve and mitogen (phytohemagglutinin-L) stimulated carp-derived leukocytes and carp brain cell line (CCB) were chosen as the highest cells reported to retain AA after dietary intake [3,4]. Cells treatment with sodium L-ascorbate was performed 2 hours before virus inoculation at concentrations 25, 50, and 75µg/ml of vitC solution in culture medium. A KHV strain (261) propagated in CCB cells was inoculated into the vitamin-treated cells at $TICD_{50}$ 10^{-2}. Ascorbic acid was re-administrated every next 24h throughout the experimental time (72h) for cell proliferation assessment and detection of reactive oxygen species (ROS). Cells were labeled for the activity of ploy caspases after 24h of a single administration of AA and KHV inoculation.

Discussed Results

Our results show that vitC performs a dual role both as antioxidant and pro-oxidative agent during koi herpes virus infection linked to the cell type. VitC protected the brain cells from damage induced by virus propagation via coordinated promotion of metabolic activity, ROS scavenging, and activation of caspases. In leukocytes, AA administration significantly enhanced cell proliferation with chemotaxis behavior relative to higher stimulation of ROS production for pathogen killing, and fluctuation in the caspase's activity related to cell death mechanism (apoptosis vs. necrosis) (Fig. 1, 2, 3).

Conclusion

The essentiality of vitC as an immune supplement in carp is highlited. Findings of this study reveal a selective antiviral/immune support machinery of vitC against KHV infection reflective of the disease-resistance influence reported in other teleost. An extra advantage is implementing vitC in in-vivo testing to assess its integral role against KHV and other infectious agents and stress-inducing substances in carp fish.

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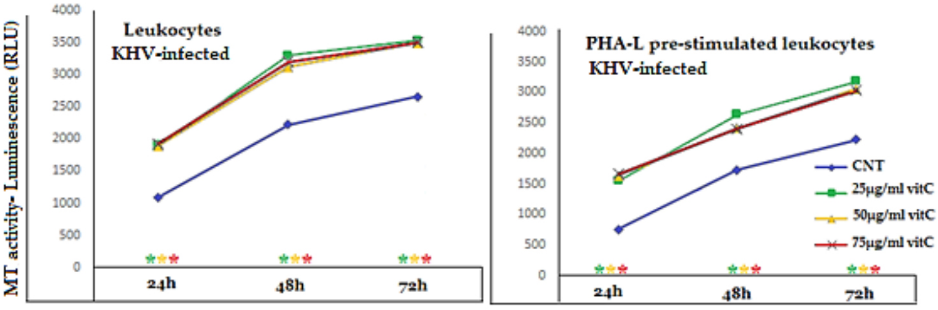

Fig. 1 Representative of cell proliferation: the metabolic activity (MT) in naive or phytohemagglutinin-L (PHA-L) pre-stimulated carp-derived leukocytes following vitC treatment (25, 50, 75µg/ml) and KHV infection (10^{-2} TICD$_{50}$). Analysis of variance for differences between controls (CNT) 0 µg/ml vitC and vitC-treated cells indicates statistically significant induction of cell proliferation *p<0.01* or *p<0.05*. RLU= relative luminescence unit.

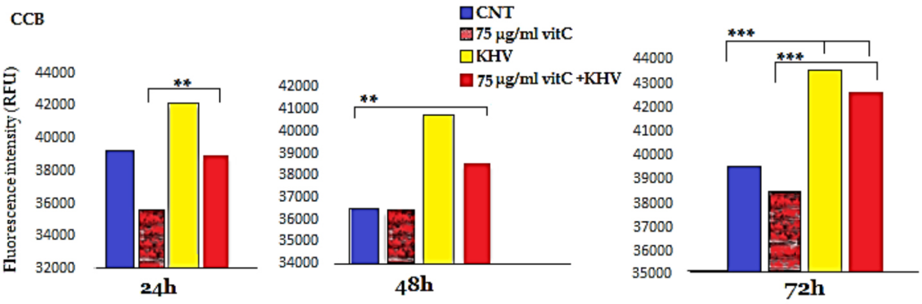

Fig. 2 Representative of ROS production: Intracellular ROS production in vitC-treated CCB cells (75 µg/ml) in the absence and presence of KHV. Data analysis is performed by t-student test for paired samples; *p < 0.05* or *p< 0.01*. RFU= relative fluorescence unit.

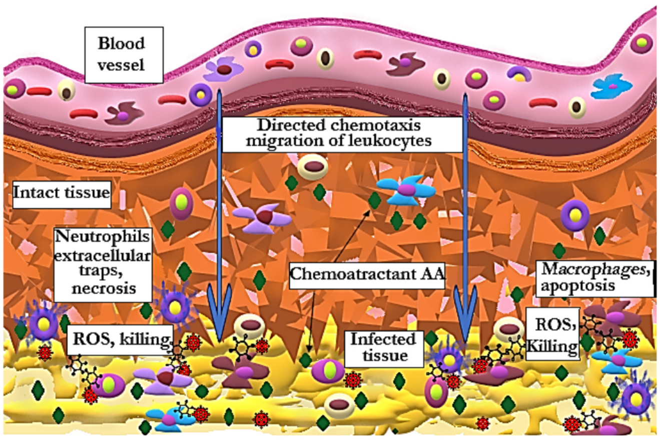

Fig. 3 VitC pro-oxidative and chemoattraction functions in immune cells: AA provokes leukocytes migration and enhances ROS production to facilitate pathogen killing via macrophage phagocytosis (apoptosis) or stimulating neutrophils' extracellular traps (necrosis).

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COMPUTER VISION BASED COUNTING OF SHIRMP IN REAL FARM CONDITIONS

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Introduction

Counting shrimp in a farm environment is challenging. Environmental factors may negatively affect image quality and make identification of individuals difficult, while overlapping individuals create a problem for many object detection approaches. Recent approaches such as bounding box annotation have resulted in high counting efficiencies in lab environments (<30 ind. m^{-2}) but failed in farm conditions (>80 ind. m^{-2}). The objective of the study was to test the density map approach to overcome the existing problem of object counting in real farm environments, i.e. when high numbers of overlapping individuals must be distinguished by computer vision. If successful, accurate automated counting will improve animal welfare and reduce working hours of farm staff so far dedicated to sampling and manual counting.

Material & methods

Videos were recorded with an iPhone 12 mobile camera (resolution 1920 x 1080) at Förde Garnelen GmbH & Co. KG farm at different light conditions, (dark and bright), at different heights (60cm, 100cm, 125cm, and 185cm) and with different types of nets (coarse, fine, for shrimp to rest on). To generate automated counting training examples, we extracted frames from each video with a step of eight frames, to avoid duplicates. Single point annotation was carried out in a CVAT format. Two models were used for accurate shrimp counting, FCRN (Full Convolutional Regression Network) (Xie, Noble, & Zisserman, 2018) and U-Net (Ronneberger, Fischer, & Brox, 2015). Of 887 images annotated, the total number of shrimp present in all images was 50319. The maximum number of shrimps in an image was 272 and the minimum number of shrimps in an image was 14. Truthing or accuracy testing was conducted with a unique dataset (images taken from different videos). Out of 90 test images, the total number of shrimps present in all images was 14715, with a maximum of 266 and a minimum of 66 individuals. Ground truth labels were generated by convolving exiting dot labels (x,y coordinates) with a Gaussian kernel. Models were trained on ground truth labels as a supervised machine learning problem. Density map output was a yellow dot for each detected shrimp.

Results abstracts

The mean absolute accuracy at different heights was statistically significant ($P < 0.01$, with highest accuracy (96) % at 185 cm height followed by 90 % and 71 % at 125cm and 100 cm height respectively (Fig. 1).

Discussion and conclusion

To our knowledge present results demonstrate for the first time the possibility of reliably counting shrimp in a commercial farm environment with multiple overlapping individuals. Counting accuracy factors such as object augmentation, model type, background contrasts will be presented along with a cataloguf of conditions (e.g. shrimp size, image, resolution) currently under investigation which need to be improved for successful use in commercial shrimp farming.

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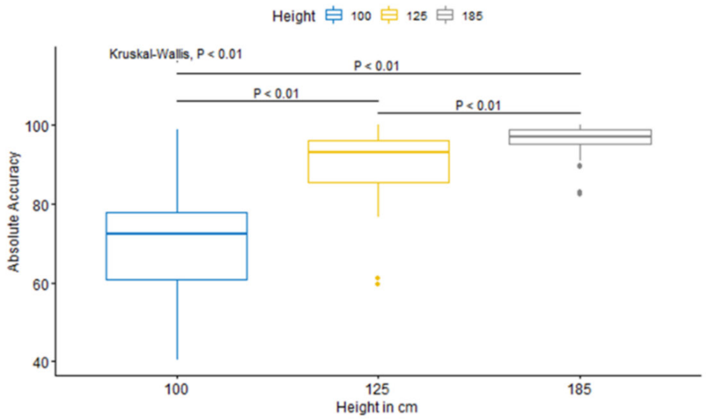
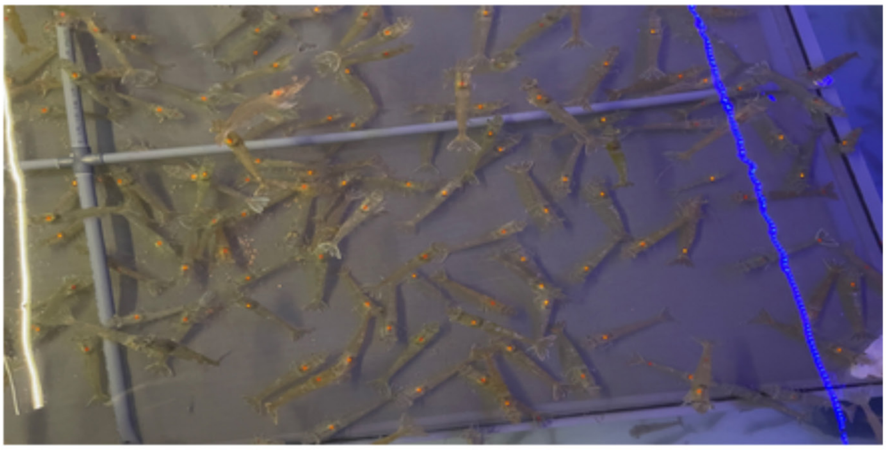


Figure 1: The influence of the camera (smartphone) height (above the pool water surface) on the error rates and counting accuracy.



Shrimp Detections

Density Map

Figure 2: Example of density map image output using the online demo version @ https://www.awi.de/forschung/besondere-gruppen/aquakultur/aquakulturforschung/projekte/monitorshrimp.html)

VALORISATION OF AQUACULTURE VALUE-CHAINS IN THE PRODUCTION OF GILTHEAD SEABREAM JUVENILES

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Introduction

Aquaculture industry is a key animal production sector to secure the future food demand. Expanding the number of raw materials will increase flexibility in the formulation of highly nutritious aquafeeds and the sustainability of the sector. The inclusion of mussel meal originated from mussel aquaculture side-streams in aquafeeds will contribute to the valorisation of aquaculture value-chains and to the circular economy approach in the industry.

The present study aimed to evaluate gilthead seabream (Sparus aurata) juveniles growth performance, feed utilisation, nutrient apparent digestibility, and nitrogen outputs to the environment when fed experimental diets with mussel meal as the main source of marine-derived protein.

Material and Methods

Three practical diets were formulated to be isonitrogenous (crude protein, ~48.6%), and isoenergetic (gross energy, ~ 21 MJ/kg). The Commercial diet (CTRL) was formulated to be similar to a commercial feed used for gilthead seabream juveniles, with fishmeal as the main source of marine-derived proteins. The other two diets were formulated to reduce dietary fishmeal inclusion in 50% (Mussel 50) and in 100% (Mussel 100) using mussel meal as the source of marine-derived protein.

Gilthead seabream juveniles (initial body weight ± 5.0 g) were acquired from a commercial aquaculture and transported to the CCMAR facilities at Ramalhete Experimental Research Station (Faro, Portugal). Triplicate groups of 90 seabream juveniles, with a mean initial body weight of 8.0 ± 0.1 g were fed one of the three diets for 8 weeks. Fish were reared in 500 L tanks, supplied with flow-through seawater (temperature: 21.8 ± 1.6 °C; salinity: 37.3 ± 0.4 psu, dissolved oxygen above 80% of saturation). Fish were fed to apparent satiety, by hand, three times a day. Feed intake was recorded, and utmost care was taken to avoid feed losses. Fish were individually weighed at the beginning, bulk weighed every three weeks and at the end of the trial, following one day of feed deprivation.

Fifteen fish from the initial stock and 18 from each replicate tank at the end of the trial, were individually weighed and measured, to calculate the condition factor. From these, three fish were stored at -20 ºC until subsequent analysis of proximal composition, in other three fish liver and posterior intestine were sampled for gene expression and microbiota analysis, and from the other 12 fish, plasma, liver and whole-fillet samples were collected for analysis of immune and biochemical parameters, antioxidant status, metabolic enzymes, proximal composition and amino acid content; liver, viscera and visceral fat were weighed to calculate hepatosomatic, viscerosomatic and perivisceral fat indexes.

The apparent digestibility coefficients of the dietary components were determined by the indirect method, with diets containing 0.02% yttrium oxide as inert tracer. Triplicate groups of fish (mean initial body weight of ± 66.8g) were allocated to cylindroconical fibre glass tanks of 100 L coupled with faeces collectors. Fish were allowed to adapt to the new conditions for ten days before starting faeces collection. During the adaptation period fish were fed by hand to apparent satiety twice a day one of the experimental diets and continue to do so throughout the trial. Tanks were thoroughly cleaned to remove any uneaten feed. Before feeding, faeces were collected daily, left to settle and water was decanted. Faeces were frozen at -20 ºC and freeze-dried prior to analysis.

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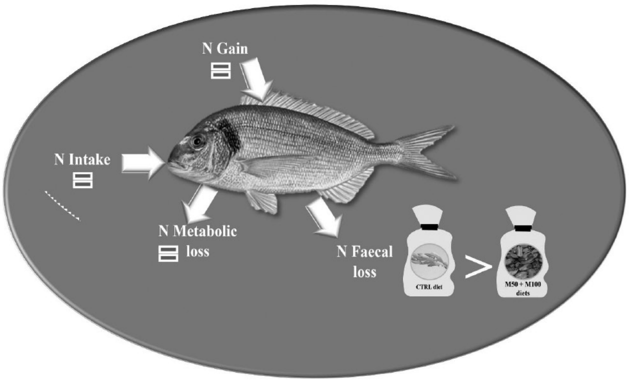

Figure 1. Daily nitrogen balance of seabream juveniles fed the experimental diets (Mussel 50 and 100) compared with fish fed the Commercial diet, at the end of the trial.

Results and Discussion

The partial or total replacement of fishmeal by mussel meal had no detrimental effect on key growth performance indicators ($p > 0.05$). All the fish (CTRL, M50 and M100) increased the initial body weight in almost 6-fold during the feeding period. Only the hepatosomatic index (HSI) presented some changes. Fish fed the diet M50 and M100 presented a higher HSI that CTRL fish ($p < 0.05$). The replacement of fishmeal by mussel meal had no impact on diets digestibility ($p > 0.05$). Fish protein, fat and energy retention were not affected ($p > 0.05$) by the dietary treatments. Phosphorous retention was lower in M100, when compared to CTRL fed fish ($p > 0.05$). Whole-body composition analysis combined with information on ADC of diets allowed the calculation of daily nitrogen and phosphorus balance. Daily nitrogen gain and metabolic losses were unaffected ($p > 0.05$) by the dietary treatments (Fig. 1).

Faecal losses were positively affected by the inclusion of mussel meal since both experimental diets (M50 and M100) presented lower values than CTRL diet ($p < 0.05$).

More parameters, such as biochemical, immune, metabolic and antioxidant responses are under analysis. Based on growth performance, feed utilization, diet digestibility, nutrient balances data, the replacement of fishmeal by mussel meal might be a viable strategy to tackle the identified technical challenge.

Acknowledgements

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SWIMMING ACTIVITY MODULATES PRO-INFLAMMATORY GENES IN GILLS AND HEAD-KIDNEY OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Exercise promotes health in mammals (Pascoe et al., 2014). In farmed fish, the physiological effects of swimming activity are, however, overlooked. Swimming is a fundamental behavioral element contributing to the fitness of most fish species. Reduced swimming performance has been associated with lower disease resistance and poorer welfare (Castro et al., 2013). The current study aims to fulfill the lack of knowledge on swimming physiology by evaluating the impact of induced swimming activity on the welfare of gilthead seabream (*Sparus aurata*).

Materials and Methods

Forty eight seabream (total length: 13.3 ± 0.3 cm; total weight: 57.9 ± 1.8 g) were individually subjected to a 6-h exercise in a swimming tunnel on continuous or discontinuous flow patterns, as follows: i) continuous low (CL, 0.8 BL s^{-1}); ii) continuous high (CH, 2.3 BL s^{-1}); iii) discontinuous low (DL, 0.2-0.8 BL s^{-1}); iv) discontinuous high (DH, 0.8-2.3 BL s^{-1}) velocities and v) control non-exercised group (CTRL). At the end of the trial, fish were euthanized, and gills and head-kidney (HK) were sampled and frozen at -80ºC. Total RNA was extracted, purified, and complementary DNA (cDNA) was synthesized from 1 µg of RNA using reverse transcriptase enzyme SuperScriptIV with an oligodT18 primer. Expression of inflammatory (*tnf-α*, *il-1β*, *il-6,* and *nf-κb*) and immune-related genes (*igf-1*, *ctsd,* and *lyz*) were analyzed by RT-qPCR and normalized with elongation factor 1 beta (*ef1β*). Results were expressed as mean ± standard error of the mean (SEM) and data were analyzed by Two-way ANOVA followed by Tukey's multiple comparison test to determine differences between the type of flow and velocity. The level of significance used was $p < 0.05$.

Results and Discussion

TNFα (tumor necrosis factor alpha), IL-1β (interleukin 1 beta) and IL-6 (interleukin 6) are cytokines considered important components in the inflammatory response in fish and are key molecules in the immunomodulatory cascade in fish gills following an environmental challenge and regulating the host defense against pathogens mediating the innate immune response (Secombes et al., 2001). Additionally, NF-κB (nuclear factor kappa b) is a transcription factor with a fundamental role in the immediate early pathogen response and in promoting inflammation (Cheng et al., 2014). The expression of *il-1β*, *il-6,* and *nf-κb* genes in gills were up-regulated in fish swimming in DL velocity condition when compared to CTRL and DH velocity conditions. However, no significant variations were found in the relative expression of immune-related genes in the HK. Higher mRNA levels of *il-1β* and *il-6* genes in HK of fish swimming in DL and CL patterns may suggest that a longer swimming period at low velocity may be required to induce significant differences compared to CTRL. Results suggest that swimming condition at DL velocity triggers in the gills a similar response to the inflammatory process and reveals how this load may regulate the immune response in this tissue. Although the relative expression of *igf-1* and *ctsd* genes in gills were increased, they did not display significant changes between experimental groups. IGF-1 (insulin-like growth factor-I) and CTSD (cathepsin D) are involved in somatic growth and innate immune response respectively (Mommsen, 2001; Subramanian et al., 2007). It is plausible to infer that a longer swimming period may require to be applied to induce significant differences in the mRNA levels for both genes.

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Conclusions

Induced swimming activity under a discontinuous low velocity may stimulate an inflammatory response in the gills of gilthead seabream. This suggests that the gills may play a key role in the immune response in addition to the well-described functions (gas and ions exchanges). It is plausible to infer that swimming conditions, often overlooked in aquaculture, may be correlated to how farmed and wild fish cope differently with immune challenges. Considering the current results, induced swimming may be an adequate tool for improving the health and fitness of farmed fish.

Acknowledgments

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DIETARY TRIBUTYRIN SUPPLEMENTATION ENHANCES THE IMMUNE AND ANTIOXIDANT RESPONSES OF RAINBOW TROUT (*Oncorhynchus mykiss*) WITHOUT CHANGES IN FISH PERFORMANCE

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Introduction

The use of dietary tributyrin (TB) supplementation in land-farmed animals has been well studied, showing the potential to improve animal performance and productivity. In contrast, its applicability in aquafeeds is less studied. Previous studies have shown that TB supplementation in fish and shrimp diets seems to alleviate some of the negative effects of high inclusion levels of plant ingredients. The beneficial effect appears to be associated with the release of butyric acid, a short-chain fatty acid composing TB, into the intestine (Abdel-Latif et al., 2020). Assessing the effects of dietary TB supplementation on plant-based diets is key for optimizing feed formulations, as TB could be used to enhance fish production and animal welfare. This will be particularly interesting for carnivorous species such as rainbow trout (*Oncorhynchus mykiss*), requiring a high substitution of fishmeal by plant-based proteins aiming to implement sustainable diets. This study investigated the potential use of TB supplementation in rainbow trout diets at different levels, aiming to improve fish performance, and studying changes in several parameters related to the innate immune and antioxidant responses.

Materials and Methods

Four hundred eighty juvenile trout (19.0±0.4 g, mean±SD) were randomly distributed in 12 tanks (210 L, 40 fish per tank) connected to a RAS system under controlled conditions and daily monitored (16±1oC; 0.30±1‰; pH 7±1; >95% air saturation; 12 h:12 h photoperiod). Water ammonium and nitrite levels were below 0.05 mg mL-1 and 0.5 mg mL-1, respectively.

Four experimental diets were formulated and manufactured by Sparos (Portugal), consisting of a basal diet (control) including 10% fishmeal and 70% plant-based ingredients (44% crude protein, 18% crude fat, gross energy 21.8 MJ kg^{-1}) with increasing inclusion levels of 0.1, 0.2 and 0.4 % of a product containing 55% TB (Lucta S.A.) in replacement of silica. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed twice a day to apparent satiety at 9:00 h and 16:00 h. Fish performance was evaluated after 44 days. Nine fish per treatment were sampled after 3 h or 24 h of feeding (absorptive or post-absorptive states, respectively) to evaluate the impact of dietary TB supplementation on innate immune parameters in plasma and antioxidant response in the liver.

Results and Discussion

Experimental diets had no significant impact on final body weight (g), weight gain (g), daily growth index (%), feed intake (g), or feed conversion ratio of rainbow trout after 44 days of feeding ($P>0.05$, One-way ANOVA). Similarly, experimental diets had no significant impact on body proximal composition (dry matter, protein, lipid, and ash contents) at the end of the growth trial ($P>0.05$, One-way ANOVA).

Regarding immune-related parameters analyzed in plasma, lysozyme activity was higher at 3 h post-feeding in the group fed the 0.2% diet compared to fish-fed control or 0.4% diets (P=0.021, two-way ANOVA). In addition, plasma antiprotease activity was higher at 24 h post-feeding in the groups fed the 0.2% and 0.4% diets compared to fish-fed the control diet ($P=0.010$ and 0.013, respectively, two-way ANOVA). Other immune-related parameters investigated in plasma, including peroxidase and protease activities, were not affected by the diets ($P>0.05$, two-way ANOVA).

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Increased hepatic catalase activity was detected in the group fed the 0.4% diet compared to trout fed the control or 0.2% diets at 3 h post-feeding (*P=0.016* and *0.019*, respectively, two-way ANOVA), suggesting an improved antioxidant response. The remaining parameters investigated in the liver of trout, including lipid peroxidation, total and oxidized glutathione levels, as well as glutathione-S-transferase, glutathione reductase, and glutathione peroxidase activities remained similar between dietary groups (*P>0.05*, Two-way ANOVA).

Conclusions

Although the supplementation of a TB product in diets of rainbow trout under the levels investigated did not improve growth or feed efficiency, it did not reduce feed intake. The results suggest that dietary supplementation with 0.2 and 0.4% (corresponding to 0.11% and 0.22% TB) could result in improved immune and antioxidant responses in trout, respectively.

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INDUCED SWIMMING ACTIVITY MODIFIES THE ANTIOXIDANT STATUS OF JUVENILE GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Exercise causes a systemic effect and promotes several biochemical and enzymatic adaptations, with a major impact on the liver and skeletal muscle functions. In mammals, the generation of reactive oxygen species (ROS) by exercise modifies cell-signaling pathways (Bouviere et al., 2021) and plays a beneficial role in the immune response (Scheffer and Latini, 2020). In fish, however, excessive ROS formation may lead to increased pro-oxidants and free radicals which damage cells and tissues, resulting in oxidative stress (OS) (Chowdhury and Saikia, 2020). Therefore, induced swimming activity may have positive (eustress) or negative (distress) outcomes depending on the conditions applied, a fundamental aspect to improve the welfare of cultured fish. The current study aims to fulfill the lack of knowledge on the impact that different swimming conditions may have on OS in the liver and muscle of gilthead seabream (*Sparus aurata*).

Materials and Methods

Forty eight gilthead seabreams (total length: 13.3 ± 0.3 cm; total weight: 57.9 ± 1.8 g) were individually subjected to a swimming trial for 6 h on continuous or discontinuous flow patterns, as follows: i) continuous low (CL, 0.8 BL s^{-1}); ii) continuous high (CH, 2.3 BL s^{-1}); iii) discontinuous low (DL, 0.2-0.8 BL s^{-1}); iv) discontinuous high (DH, 0.8-2.3 BL s^{-1}) velocities; and v) control non-exercised group (CTRL). Fish were euthanized at the end of the trial. Liver, white and red muscles were sampled and immediately frozen at -80ºC. The OS parameters in liver and muscle, including lipid peroxidation levels (LPO), superoxide dismutase (SOD), catalase activity (CAT), glutathione S-transferase activity (GST), glutathione reductase activity (GR) and both oxidized glutathione (GSSG) and total glutathione levels (tGSH) were determined. Results were expressed as mean ± standard error of the mean (SEM) and data were analyzed by Two-way ANOVA followed by Tukey's multiple comparison test to determine differences between the type of flow and velocity. The level of significance used was $p < 0.05$.

Results and Discussion

The glutathione system (GPx, GST, tGSH, and GR) and enzymes including SOD and CAT are key components of the antioxidant system in fish tissues (Kabel, 2014). A significant reduction of GR activity in the liver was observed across all swimming conditions compared to the CTRL group. In addition, the tGSH level in the liver increased in the CH group compared to the CTRL. In white muscle, GR activity increased significantly in both high swimming velocity groups (CH and DH), but tGSH increased only in the CH group compared to the CTRL group. On the other hand, discontinuous velocity (DL and DH) triggered an increase of CAT activity in red muscle compared to CTRL, although increased LPO level was evident only in the DH group. Even though various tissues may contribute to exercise-induced ROS production, it is expected that active muscle fibers will be the main source of ROS (Powers et al., 2020). White muscle in teleost fish is regarded as being used mainly for burst sprints, as it can contract quickly by using (anaerobic) glycolysis, whereas aerobic red muscle is used during sustained swimming (Davison and Goldspink, 1984). In this study, an increased CAT activity in red muscle was linked to a discontinuous swimming, which may indicate a need for H_2O_2 removal in this tissue, which was accompanied by a higher LPO level (DH condition). Nonetheless, an opposite pattern of CAT activity was observed in the white muscle in the discontinuous swimming, revealing that similar swimming conditions can result in different OS responses, depending on the muscle type. Finally, a reduction on hepatic GR activity across all the swimming conditions showed that exercise in fish acts as an important factor in modifying the antioxidant balance in liver.

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Conclusions

Induced swimming activity triggered different responses of antioxidant enzymes between white and red muscles, indicating that optimal swimming conditions shall be applied in this fish species to avoid OS. Thus, swimming conditions should be subjected to increasing attention in aquaculture, as it may influence fish welfare.

Acknowledgments

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POTENTIAL OF SOLID-STATE FERMENTED BREWER'S SPENT GRAIN AS A NEW INGREDIENT FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*) DIETS: EFFECT ON LIVER OXIDATIVE STATUS

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Introducion

Utilization of by-product as an aquaculture-feed ingredient has high relevance not only from a circular economy point of view but also considering that the demand for such ingredients is growing. BSG is rich in fibre and but also in protein (up to 30%) and certain phytochemicals with many biofunctions, such as antioxidant, antiatherogenic, and anti-inflammatory activities (Rachwal et al., 2020). Biotechnological processes, such as solid-state fermentation (SSF), can be effective approaches to improve BSG nutritional value and antioxidant potential by promoting the lignocellulosic cell-wall disruption and dietary nutrient/phytochemicals availability. Our preliminary results demonstrated that BSG can be incorporated at 20% in aquafeeds for carnivorous fish, namely European sea bass (Dicentrarchus labrax), after a biological treatment with SSF, without negative effects on fish growth performance (Estevão-Rodrigues et al., 2022). The focus of the present study was to assess the effect of dietary inclusion of untreated and biological-treated BSG on hepatic oxidative status of European seabass.

Materials and Methods

BSG was fermented using tray-type bioreactors for 7 days at 25°C with spores of *Aspergillus ibericus* (MUM 03.49) at a concentration of 10^6 cells mL-1. A practical diet (45% crude protein and 18% crude lipids) was used as a control and formulated to be challenging, with 15% fishmeal and a mixture of plant ingredients Four other diets were formulated similarly to the control diet, including 10% and 20% unfermented (10BSG and 20BSG diets) or fermented BSG (10BSG-SSF and 20BSG-SSF diets). The test ingredient was included in the diets at the expense of a mixture of agricultural ingredients in the control diet. Sixteen juvenile of European bass (initial body weight 49 ± 1g) were randomly distributed to each tank and each experimental diet was assigned to triplicate of these groups. Fish were manually fed, until apparent satiation, twice a day, 6 days a week, for 68 days. After the experimental period, lipid peroxidation(LP) and antioxidant enzymatic activities were assessed in the liver. The test diets were all compared to the control (orthogonal contrast) and the interaction between the incorporation levels (BSG) and the bioprocess (SSF) (orthogonal contrast) was also verified.

Results

Compared to unfermented BSG, the application of SSF to BSG contributed to increase
the crude protein content by 21% and reduce the fiber levels by 34%. Results of the feeding trial were not the aim of this study and are presented elsewhere (Estevão-Rodrigues et al., 2022). In brief, growth erformance was significantly lower in fish fed diets including unfermented BSG. However, fish fed the fermented BSG had similar growth performance (20BSG-SSF) or superior to the control (10BSG-SSF). The 10BSG diet promoted an increase in hepatic LPO, whereas control diet and the other experimental groups (20BSG, 10BSG-SSF and 20BSG-SSF) exhibited similar LPO levels (Table 1). No effects on activities of GR and CAT (except in 10BSG-SSF group that was lower) in relation to control diet were observed in the liver of fish fed the BSG-based diets. Except for 10BSG group, the G6PDH activity was significantly lower in fish fed diets with unfermented or fermented BSG than with the control diet. Among the BSG-based diets, the biological treatments (SSF) led to a decrease in all the antioxidant enzyme activities, while dietary incorporation level promoted a decrease of G6PDH activities and LPO contente.

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Table 1. antioxidant enzyme activities (CAT for U mg-1 protein; mU) and LPO (nmol MDA g-1 tissue) responses in European sea bass fed experimental diets for 68 days.

	CONTROL	**10BSG**	**10BSG-SSF**	**20BSG**	**20BSG-SSF**	**SEM**
GR[1]	4.15	3.14	2.72	4.96	4.29	2.06
G6PDH[2]	11.40	10.03	7.07	7.19	6.28	2.98
Catalase	153.98	149.52	138.11	120.46	118.93	24.82
LOP[3]	19.88	29.74	23.70	21.84	20.24	6.89

Orthogonal contrast for the effects of the experimental diets	**GR**	**G6PDH**	**Catalase**	**LOP**
Control X 10BSG				*
Control X 10BSG-SSF		*	*	
Control X 20BSG		*		
Control X 20BSG-SSF		*		
Process SSF X BSG diets	*	*	*	
Level (10) X Level (20) diets		*		*
Interacion				

Values are presented as means ± standard error of the mean (SEM) (n=9). Glutathione reductase[1]; glucose-6-phosphate dehydrogenase[2]; lipid peroxidation[3]. Orthogonal contrast: ∗ denotes a significant effect ($P<0.05$).

Conclusion

Although no alterations were observed in hepatic LPO content of fish fed the BSG diets (except in 10BSG diet) in relation to control group, the lack of induction of the antioxidant enzyme mechanism may be regarded as a sign of lower risk to oxidative stress. In addition, we cannot discard that putative beneficial effects of BSG on LPO content might have been masked due to antioxidant constituents/components present in PF of control diet. Althought further studies are required, the antioxidant compounds' bioaccessibility seemed be more modulated by the level of BSG incorporation than by the biological treatment applied to BSG as only lower LPO content were recorded in 20%BSG-based diets. Overall, the results of the present study indicate that BSG can be incorporated at 20% in aquafeeds without detrimental effects on hepatic lipid oxidative damage of European sea bass.

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FERMENTED BREWER'S SPENT GRAIN: EFFECT ON GROWTH PERFORMANCE AND LIVER OXIDATIVE STATUS FOR MEAGRE (*Argyrosomus regius*)

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Introduction

The significant inclusion of plant ingredients in the diet of carnivorous fish species can compromise growth performance, digestibility, intestinal homeostasis, immunological parameters, and disease resistance (Oliva-Teles et al., 2015). This happens due to the anti-nutritional factors and the absence of enzymes and microbial capable of degrading the plant cell wall. The solid state fermentation (SSF) process produces enzymes such as xylanase and cellulase, degrading the cell wall and making nutrients available inside the cell. The present study's focus was to assess the effect of dietary inclusion of untreated and biological-treated SSF of brewer's spent grain (BSG) on growth performance and hepatic oxidative status of meagre (*Argyrosomus regius)*.

Materials and Methods

BSG was fermented using tray-type bioreactors for 7 days at 25°C with spores of *Aspergillus ibericus* (MUM 03.49) at a concentration of 10^6 cells mL^{-1}. A practical diet (45% crude protein and 18% crude lipids) was used as a control and formulated with 15% fishmeal and a mixture of plant ingredients. Three other diets were formulated similarly to the control diet, including 10% unfermented (10BSG diet) or 10% fermented BSG (10BSG-SSF diet). The non-fermented and fermented BSG was included in the diets at the expense of the control diet plant ingredients mixture. A growth trial was performed in a RAS system with 9 tanks of 300 L. Nine meagre (initial body weight 83.1 ± 1.03g) were randomly assigned to each tank, and each experimental diet was assigned to triplicate of these groups. Fish were manually fed until apparent satiation, twice a day, 6 days a week, for 68 days. After the experimental period, growth performance, feed utilization efficiency, and hepatic oxidative status were evaluated.

Results and Discussion

BSG proximal composition was meaningly modified by the SSF process. Protein content increased by 21%, and lipid content decreased from 6 to 3%, while the lignin, cellulose and hemicellulose decreased by 7, 30, and 34%, respectively. Moreover, cellulase and xylanase activity were also detected, averaging 109 and 282 U/g of fermented BSG.

At the end of the growth trial, it was observed that the inclusion of 10% unfermented BSG significantly reduced final body weight, weight gain, daily growth rate, and feed efficiency compared with the control diet. Feed intake was also reduced by about 13%, although not significantly. Contrarily, the dietary inclusion of 10% of fermented-BSG restored these parameters to levels similar to the control diet (Table 1).

Hepatic catalase, glutathione peroxidase; and lipid peroxidation activities were not affected by the dietary treatment. G6PDH activity of fish fed the 10BSG diet was lower than that of the other diets (Table 2).

Conclusion

SSF of BSG improved its nutritional profile. As a result, fermented BSG can successfully replace 10% of a mixture of agriculture-based ingredients without compromising meagre's growth performance, feed utilization, and liver oxidative status, contrary to the non-fermented BSG.

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Table 1: Growth performance of meagre fed the experimental diets containing 10% of unfermented BSG and fermented BSG with *Aspergillus ibericus* (BSG-SSF) for 68 days.

	Control	10BSG	10BSG-SSF
IBW (g)[1]	83.1±1.03	84.4±2.02	84.5±1.90
FBW (g)[2]	152.6±4.74^{b}	134.7±3.04^{a}	157.7±4.54^{b}
WG (g/kg ABW/day)[3]	8.66±0.58^{b}	6.75±0.46^{a}	8.01±0.33^{b}
DGI[4]	1.43±0.10^{b}	1.08±0.08^{a}	1.32±0.60^{b}
FI (g/kg ABW/day)[5]	5.01±0.21^{ab}	4.36±0.25^{a}	5.13±0.36^{b}
FE[6]	1.72±0.04^{b}	1.31±0.22^{a}	1.84±0.10^{b}
Survival (%)	70.3	81.4	92.5

Values are presented as means (n=3) and standard deviation (SD). Means in the same row with different superscript letters are significantly different (One-way ANOVA; Tukey test; p<0.05). ABW: average body weight, Initial Body Weight[1], Final Body Weight[2], Weight Gain[3], Daily Growth Index[4], Feed Intake[5], Feed efficiency[6]

Table 2. Enzymatic parameters of hepatic oxidative stress (U mg-1 protein) and LPO (nmol MDA g-1 tissue) responses in meagre fed experimental diets for 68 days.

	Control	10BSG	10BSG-SSF
GR[1]	27.93±1.71	24.92±2.76	26.27±2.49
Catalase	74.02±5.20	68.80±5.64	67.61±5.76
G6PDH[2]	15.60±1.08^{b}	10.53±1.22^{a}	12.81±1.56^{b}
LOP[4]	20.46±1.3	18.57±2	23.02±2.7

Values are presented as means (n=9) and standard deviation (SD). Means in the same row with different superscript letters are significantly different (One-way ANOVA; Tukey test; p<0.05). Glutathione Reductase[1]; Glucose-6-phosphate Dehydrogenase[2]; lipid peroxidation[3]

Acknowledgment

This work was funded by the Ocean3R project (NORT-01-0145-FEDER-000064), supported by the North Portugal Regional Operational Program (NORT2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF). This research was also supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

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CHANGE AND RACE FISH MACHINE INTERACTION – EXPERIMENTAL RESULTS ON THE REACTION OF ATLANTIC SALMON *Salmo salar* WHEN EXPOSED TO EXTERNAL INFLUENCE FACTORS

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Introduction

In this abstract, results are presented from the study of the behaviour change of Atlantic salmon (*Salmo Salar*) in an industry scale fish farm when exposed to different influence factors. Data is gathered to study the fish behaviour change when exposed to structures with varying shape, movement, and sound. The fish reaction to the structure has been studied during static and moving case studies to identify and quantify the distance that the fish keep from the different structures and how the fish reacts when the structures are moving. Furthermore, to study how sound affects the fish behaviour, underwater speakers have been places inside the structures, exposing the fish to frequencies between 100 and 1000 Hz.

This work was financed by the Research Council of Norway through the project: CHANGE and RACE Fish Machine Interaction [1].

Methods

Two yellow structures (cube and cylinder) with diameter/side length of 0.6 meters was equipped with cameras and echo sounders and lowered to eight meters depth. The cameras and sensors were GOPros and BlueRobotics Ping360 echo sounders, which have a sampling rate of 9 seconds per 360° swipe when set to a distance of 2 meters. Four replicates of videos and echo-sounder data have been obtained from two cages in an industrial scale fish farm which is part of SINTEF ACE [2] during field trials in 2021.

When studying the fish reaction to moving the structure, the structure was manually pulled 10 meters over approx. 20 seconds using ropes, with 10 minutes between each movement. During the audio tests, sounds were played for 60 seconds at a time with a 10-minute interval in between. Sounds of 100 Hz, 200 Hz, 400 Hz, 600 Hz and 1000 Hz were played in a random order, and each frequency was played several times.

Results

The sonar data shown in Figure 1a show fish presence in a radius of 5 meters around the stationary structure over a period of 10 minutes. Based on sonar data from the field trials, the fish seem to keep a distance of approx. 2 meters to the structure when the structure is stationary. Similar results were obtained from the movement tests without audio, with the fish still keeping approx. 2 meters distance, see Figure 1b. These results were similar for the cylindrical structure, which was also tested.

The videos from the audio experiments have been analysed to observe and document the behavioural change of fish. From viewing the data it seemed evident that the fish showed no reaction when playing audio of 100 Hz, 600 Hz or 1000 Hz. For 200 Hz and 400 Hz, however, the fish react differently. Before the audio is played the fish can be seen swimming past the structure at a distance. The fish then has a clear reaction, as shown in Figure 2: When the audio starts playing, the fish swims away and disappears from the view of the cameras. Most fish stay away for the duration the frequencies are playing and do not resume undisturbed schooling behaviour until after the sound has stopped. An exception to this is if the audio is played during feeding: if this is the case the fish exhibit no response to the signal, seemingly only focused on feeding. Because of the relatively low sampling rate of the echo sounders, it is difficult to use this data to analyse the immediate reaction of the fish to the frequencies.

Conclusion

In conclusion, Atlantic salmon seemingly change behaviour when exposed to audio signals with a frequency between 200Hz and 400Hz. The fish stays away, slowly returning only after the signal has stopped. The presence of a static structure, and a structure moving in the environment without sound, does not seem to trigger this immediate response. The fish keep an average distance of 2 meter from this structure when static and move gradually out of its path when the structure is moving. The distance kept from the structure may relate to the structure size, but more tests need to be executed to confirm this. In addition, the results indicate that the salmon did not react differently to structures of different shapes when the size and colour stayed the same.

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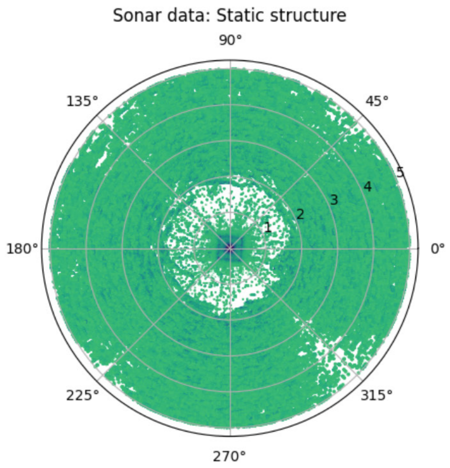

Figure 2a: *Sonar data of stationary, cubic structure. The fish seem to keep a fixed distance of approx. 2 meters.*

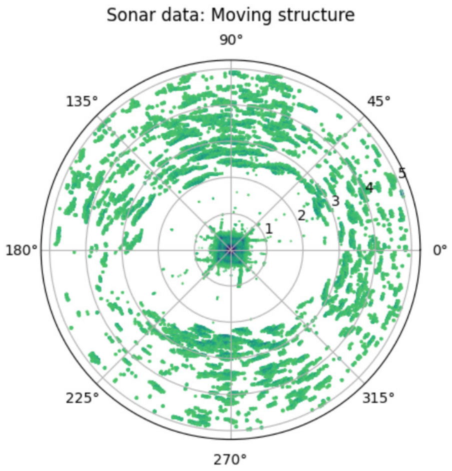

Figure 2b: *Sonar data of moving, cubic structure. The fish seem to keep the distance of approx. 2 meters even during movement.*

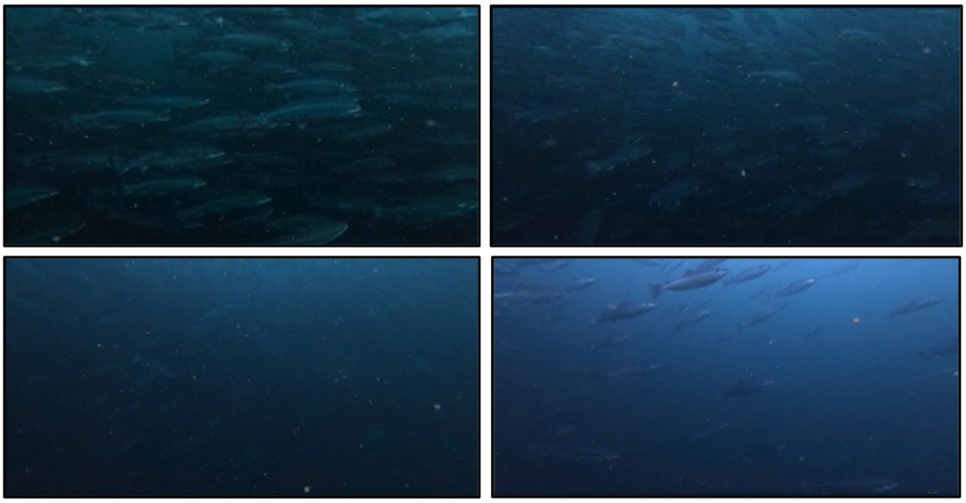

Figure 2: *Images from video when playing sound of 400 Hz for 60 seconds. From top left to bottom right: Still photos taken 2 seconds before sound started playing, the moment the sound started, 4 seconds after audio started playing, and 30 seconds after audio started playing. Fish reacts strongly when audio starts playing, and most of the fish stays away as long as the audio is still playing.*

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GENOMIC RELATIONSHIPS BETWEEN TRAINING AND TESTING SETS AFFECT GENOMIC PREDICTION ACCURACY OF NODAVIRUS RESISTANCE IN GILTHEAD SEA BREAM

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Introduction

Gilthead sea bream (*Sparus aurata*) has been reported to be susceptible to a reassortant betanodavirus strain (RGNNV/SJNNV), posing a new threat for sea bream industry (Volpe et al. 2020) and raising the attention to selective breeding as a plausible disease prevention action. Genomic selection might be beneficial for traits, such as disease resistance, characterized by difficult, expensive and time-consuming routine individual phenotyping. Genomic models are trained firstly using a reference population of full- and half-sibs of the future breeding candidates, but, in a long-term view, the prediction of the genetic merit of future breeding candidates should be satisfactory even when the reference population consists of distant relatives of the animals to be predicted. In this sense, the genomic predictive accuracy provided by random k-fold cross-validations might be unrealistic. In this study, we assessed the accuracy of a genomic prediction model for VNN symptomatology pseudo-phenotypes (estimated breeding values, EBV) in gilthead sea bream in three different validation settings: 1) a random cross-validation; 2) a cross-validation based on genomic clustering; 3) a leave-one-family-out (LOFO) validation focused on the parents of the fish subjected to the VNN challenge test.

Materials and methods

The experimental fish were produced at a commercial hatchery in three independent full factorial matings (10 sires × 12 dams; 10 sires × 7 dams; 10 sires × 5 dams). At the age of 19 d post-hatching, 972 larvae were subjected to a challenge test by tank immersion using the RGNNV/SJNNV strain; the symptomatology was recorded as a binary trait. The experimental fish and their parents (22 dams and 28 sires) were genotyped using the Med_Fish SNP array (Peñaloza et al. 2021; 26,591 SNPs after quality check and imputation). The SNP genotypes were used as predictors of EBV for VNN symptomatology by means of a Bayesian Ridge regression (BRR) model. The accuracy of the model was assessed in three different validation settings: 1) a 4-fold random cross-validation; 2) a 4-fold cross-validation based on genomic clustering, where four groups (336, 177, 328 and 133 individuals, respectively) were created using K-means clustering based on genomic relationships to reduce the relatedness between training and testing populations (relatedness between one group and the others: -0.07, -0.06, -0.03, -0.04, respectively), while maximizing the relatedness within each group (genomic relatedness within group: 0.12, 0.24, 0.04, 0.26, respectively); 3) a LOFO validation, where the BRR model was trained on the EBV of all animals except the offspring of the sire (or dam) used one-at-a-time as an independent test set. The Pearson product-moment correlation (r) between the observed and predicted values was computed as a measure of prediction accuracy for the three validation procedures.

Results

Genomic predictions of EBV for VNN symptomatology showed high accuracy in random cross-validation ($r = 0.90$; Figure 1a). The predictive accuracy decreased when genomic relatedness between training and testing sets were minimized ($r = 0.53$; Figure 1b) and resulted very low in the LOFO procedure ($r = 0.13$; Figure 1c).

Discussion

In gilthead sea bream, the genomic prediction of the EBV as pseudo-phenotypes for VNN symptomatology led to a very high accuracy in a random 4-fold cross-validation setting; however, to obtain a more realistic insight, a 4-fold cross-validation based on genomic clustering and a LOFO validation were developed. The accuracy obtained in the validation procedure based on genomic clustering was lower than that of the random cross-validation, but still satisfactory, suggesting that in a scenario where the reference population consists of distant relatives of the animals to be predicted, the predictive performance of such model would be acceptable. This is of great interest in an applicative context, particularly when considering traits phenotyping difficulties and complexity. Conversely, in the LOFO procedure, the accuracy dropped remarkably (-85.6%), suggesting that the training of the genomic model was based on data of animals weakly related to the target individual. In fact, the parent contribution to offspring was rather unbalanced, with 3 dams out of 22 producing almost 67% of the individuals, and 6 sires out of 28 producing almost 48% of the individuals.

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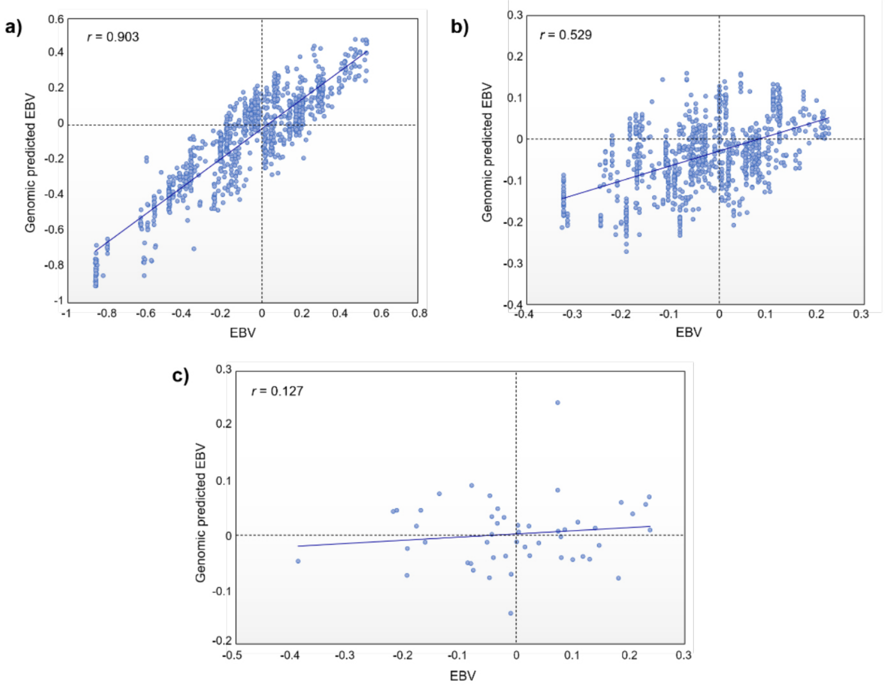

Figure 1. Relationships between EBV and their genomic predictions obtained in: (a) a random 4-fold cross-validation; (b) a 4-fold cross-validation based on genomic clustering; (c) a leave-one-family-out validation focused on the parents of the challenged fish. r: Pearson product-moment correlation.

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EVALUATION OF IMTA-PRODUCED MACROALGAE (*Ulva lactuca*) MEAL AS DIETARY INGREDIENT IN FEED PELLETS FOR SOUTH AFRICAN ABALONE (*Haliotis midae*)

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Introduction

Abalone is one of the most valued and highly sought-after seafoods globally with South Africa being the largest producer of farmed abalone outside Asia. They are cultivated in flow-through raceway tanks in South Africa and fed fish and soy meal protein-based pellets or a combination of pellets and live macroalgae until they reach market size. Research is underway to substitute the fish meal component with a less expensive and more sustainable protein sources. The seaweed *Ulva* is cultivated as part of an intergrated multi-trophic aquaculture (IMTA) in the effluent water of several commercial abalone farms, where it acts as a biofilter to remove dissolved waste nutrients and harvested as a fresh abalone feed supplement. Effluent-grown *Ulva* can be an excellent source of vitamins, minerals, alanine, glutamic acid, iron, soluble fibers and with protein levels of up to 40%. In the present study we evaluated the inclusion effect of nutrient-enriched macroalgae (*Ulva species*) meal in a pelleted abalone diet.

Materials and methods

Ulva meal, experimental diets preparation

U. lactuca was cultured on a commercial abalone farm for 30 days using a 308.57 m3 (36.735 x 8.4 x 0.70 m) D-ended paddle-wheel biofilter pond which received effluent from abalone production raceway tanks upstream. The abalones supplying the effluent were daily fed a combination diet of commercial feed pellet (Abfeed™ S34, i.e., 34% protein, 4.5% lipid) and fresh macroalgae (*Ulva* and *Gracilaria sp*). At the end of the production cycle of the *Ulva*, it was harvested, rinsed in fresh seawater, and dried at 40 °C in a locally fabricated drier. The *Ulva* meal was subjected to biosecurity measures before being included as a feed ingredient in pelleted diets manufactured by at a commercial abalone feed company (Marifeed Pty Ltd, South Africa). Six isonitrogenous experimental diets containing 34% protein were prepared to contain graded levels of IMTA *Ulva* meal ranging from 0% – 12% of the dry mass. The *Ulva* meal replaced protein from fish and soya at 0% (basal diet), 0.75%, 1.5%, 3%, 6%, and 12%.

Feeding trial

The diets were fed daily to triplicate groups of grow-out (35-45g) *H. midae* for 244 days (December 2020–August 2021). Randomly selected abalones per treatment (n=90) were sampled for shell length and weight at stocking, after 4 and 8 months of intensive feeding. Triplicate samples of abalone for each dietary treatment were collected at the end of the trial and analysed for their proximate compositions. The daily increase in shell length, weight, specific growth rate, body weight to shell length ratio, feed conversion ratio, protein efficiency ratio and the energy efficiency ratio of cultured abalones were measured and calculated at the end of the experiment.

Results

After eight months of feeding, the weight and shell length of abalone across the various feed treatments had improved substantially as their growth and nutrient utilisation were influenced by the dietary inclusion of IMTA *Ulva* meal in the diet. Feed consumption significantly improved ($p<0.05$) with an increasing level of *Ulva* meal in the pellet ($F_{(5, 12)} = 10.242$, $p= 0.0005$). The best apparent feed conversion (1.18 ± 0.11) was recorded for abalone fed 1.5% IMTA *Ulva* inclusion diet while the lowest (2.12 ± 0.28) recorded at 12% inclusion. There was no significant difference ($p>0.05$) in the final weights, shell length and weight gains of the animals up to 12% inclusion of *Ulva* meal in the diet. However, the highest weight gains were recorded at 3%-6% inclusion of *Ulva*. The results obtained in this study suggest that the use of IMTA-produced *Ulva* meal in the diet for South African abalone (*H. midae*) could be promising as no adverse effect on growth or body composition was observed.

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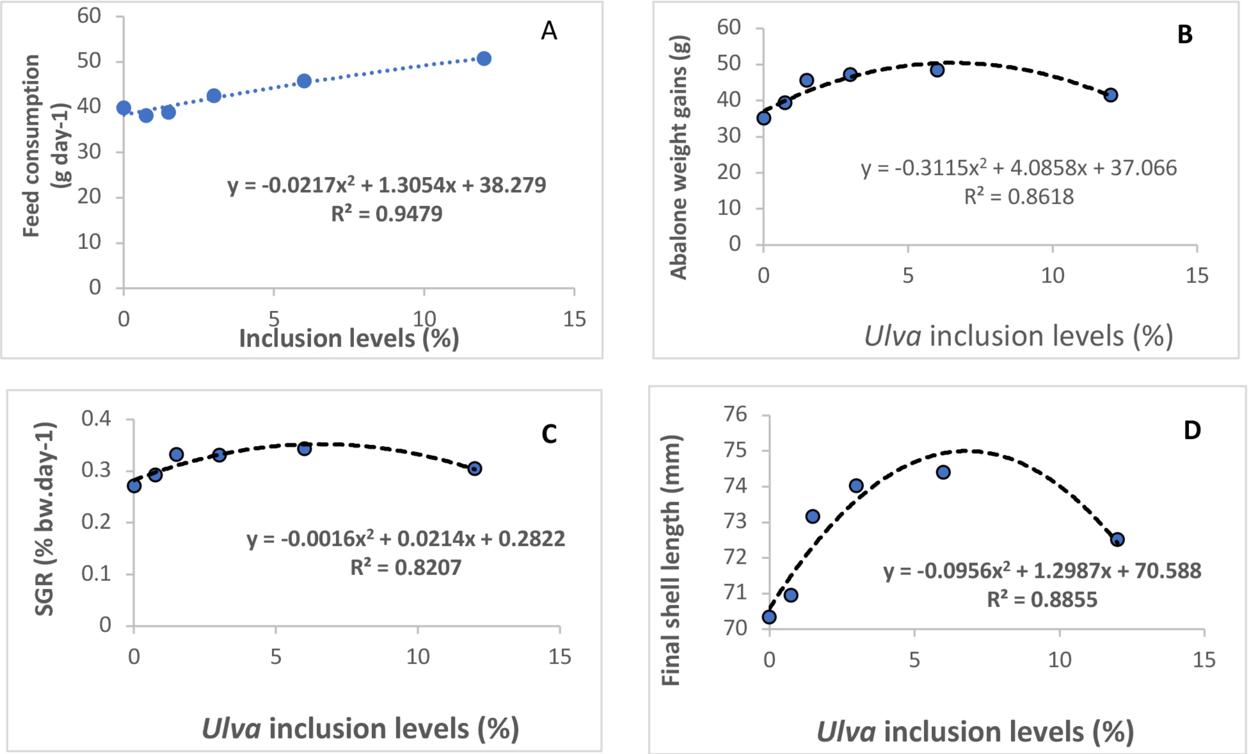

Figure 1: Polynomial regression of abalone feed consumption (A), weight gain (B), specific growth (C), and final shell length (D) against inclusion levels of IMTA *Ulva* in commercial pellet fed to the animals.

CLIMATE CHANGE AND AQUACULTURE: WORKING ON A PUZZLE WITHOUT ALL THE PIECES

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Introduction

Climate change has, and continues to have, widespread negative impacts across the world. Some effects are now irreversible, and there are situations where loss and damages are unavoidable, but there is still potential to reduce exposure and vulnerability to aspects of climate change through adaptation (IPCC, 2022). Aquaculture has a range of adaptation responses that could be implemented, each with their own advantages and disadvantages. However, therein lies the question – how can we identify the most appropriate adaptation responses for aquaculture? Unfortunately, there is no simple answer.

There are many uncertainties about the future, and information is lacking on how different specific locations, species, and farming systems will be affected. The aquaculture industry and policymakers will need to make decisions on future strategies despite incomplete and missing information, which may lead to the wrong decisions being made. At the same time, waiting for impacts to occur before making any strategies is also dangerous. Many adaptation responses require time to plan, develop, and implement. Reactionary approaches would leave many parts of the sector vulnerable and unable to respond effectively, threatening aquaculture production.

This may seem overwhelming, and some may perceive this as an impossible challenge. However, whilst it is very difficult to accurately predict the future, there are ways that researchers, industry, and other stakeholders, can work together to establish supporting knowledge that can increase the confidence in decision-making. Here we present a structured approach to support optimisation of existing knowledge, prioritisation of new knowledge generation, and knowledge translation towards improved decision-making. Salmon aquaculture in Norway is used as a case study.

Methods

The first step involved developing a knowledge base with information on how climate change may affect Norwegian salmon aquaculture (Falconer et al., 2022). The scope covered the production stages from breeding to harvest and processing, as well as components of the wider supply chain such as transportation and feed. The next step involved framing the knowledge base within local-scale assessment. Models and *in-situ* data were used to provide context for more site-specific analysis.

Results and Discussion

In the knowledge base, 7 climate stressors, 45 impacts, and 101 adaptation responses were identified, as well as knowledge gaps where further research was needed. Identified impacts included damage to land-based infrastructure and transport routes, health challenges, product quality issues, and changes in availability of feed ingredients. Most of the impacts were linked to multiple stressors, highlighting the need for further research into their interactions and combined effects. This knowledge base provided a starting point that was then used to guide the local-scale assessment. There were some challenges in moving towards more detailed local-scale assessments, particularly around data-availability. However, it was still possible to integrate contextual information that enabled an overview of what potential future challenges could be.

Conclusion

Climate change and aquaculture is a puzzle, but it is one that cannot be ignored. There are many complexities, and generalisations can be misleading. Broad-scale assessments are a starting point but need to be distilled to local-scale using contextual information. This then provides a more realistic overview of the climate change puzzle at farm-level and a route forward to support long-term decision making.

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Acknowledgements

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EVALUATION OF APPLYING ENVIRONMENTAL ENRICHMENT ON STERLET (*Acipenser ruthenus* L.) IN EARLY LIFE STAGES

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Introduction

Nowadays, nature conservation programs are ongoing to maintain sturgeon populations through ex situ interventions that place great emphasis on artificial reproduction. Despite the partial recovery of populations due to annual restocking and improving water quality of upper and middle Danube and some of its tributaries, the sterlet *Acipenser ruthenus* is still considered Vulnerable by the IUCN (Kubala et al. 2021). Generally, restocking programs mainly focused on the impact of genetic processes on wild populations, however, the conditions of the rearing environment of fish for restocking purposes is a subject raising attention. Rearing fish in a commercial aquaculture manner may lead to decreased post-release survival due to artificial rearing environments that may induce behavioral responses different from those considered normal in wild fish (Williams et al. 2009; Johnsson et al. 2014). Manipulation of the rearing environment may have an impact on neural plasticity and cognition (Salvanes et al. 2013), and also on growth parameters and survivorship of fish and subsequently, it can improve welfare and fitness-related traits (Carrera-García et al. 2016; Lee et al. 2018). The aim of the current experiment was to evaluate the effect of environmental enrichment on the growth and survival of sterlets in early life stages.

Material and methods

Fish specimens used in the trial were obtained via artificial propagation of pond-reared sterlet breeders of Research Center for Fisheries and Aquaculture, MATE HAKI, Szarvas, Hungary. At 24 day post-hatch (DPH) fish were randomly divided into duplicated plain aquaria – control group (C) and duplicated aquaria with small gravel (0.5–2 cm) on the bottom – enriched group (E) for 44-day-long rearing. In total 272 fish were stocked per aquaria. Rearing conditions were tested in duplicates under natural photoperiod. Random subsamples (n=31) were collected from the aquariums and were measured for weight and total length. In total, four samplings (30, 37, 51, and 64 DPH) were conducted till the end of the rearing. Larvae were initially fed with freshly hatched *Artemia salina* nauplii and frozen bloodworms. Weaning to dry starter (BioMar Larviva 300 and BioMar Larviva 500) was initiated at 29 DPH co-fed with Chironomus sp. until the end of rearing.

Results

Significant differences were found in final body weight of fish at 64 DPH being significantly smaller in the E group (Fig.1). Likewise, the two experimental groups significantly differed in coefficient of variation in final body weight being higher in the C group. Finally, significantly higher survival (72.24 ± 2.9 % vs 89.15 ± 1.8 %) was noticed in the E group.

Discussion

Overall, our results show that environmental enrichment might play important role in the growth and survival of sterlet in the early life stage. The fish from environmental enriched groups were significantly smaller than fish from control aquariums however without influencing negatively the condition factor, implying that enriched-reared fish were perhaps forced to swim more searching for food among the gravels while in bare tanks the food was available for the fish without making a big effort. Furthermore, the mortality was significantly lower in E groups respectively indicating that the gravels might serve as a shelter for the fish during the experiment. Similar to our outcome higher survival rate was obtained in enriched groups in zebrafish (*Danio rerio*) larvae (Lee et al. 2018). In contrast with our result, the study of Carrera-García et al. (2016) in European sturgeon (*Acipenser sturio*) shows that fish in enriched conditions were bigger than fish reared in the traditional way and there was no difference in mortality between the two groups. Thus, the rearing conditions for sterlet early stages should be considered due to the potential benefits in terms of survival, and perhaps the fish may show enhanced exploratory behavior which is beneficial for stock enhancement in wild. Further studies should address the effect of environmental enrichment on stress response and anxiety-like behavior, behavioral plasticity, memory, and learning ability in sterlet.

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ASSESSMENT OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE STERLET (*Acipenser ruthenus*), FROM GENE BANK AND NATURAL POPULATIONS IN HUNGARY

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Introduction

The sterlet is the only sturgeon species in the Hungarian section of the Danube and Tisza rivers, that is still present, and has a stabile population. The *ex-situ* sturgeon live gene bank of MATE (the Hungarian University of Agriculture and Life Sciences) and HAKI (Research Centre for Aquaculture and Fisheries) has a significant role in the preservation of this vulnerable species by its conservation management practice and conservation-based restocking programs. The individuals of the species are kept in earthen pond conditions in the gene bank of HAKI. Broodstock's collection of sterlet in the gene bank started in the '80-is, by capturing wild individuals primarily from Danube, Tisza, and Körös Rivers. This collection formed the basis of the recent broodstock of the sturgeon live gene bank. In the last decade, with the aim of compensate the fish stock of the locally habitat in Hungary, were no regular stocking activities. However the restoration of genetic diversity were taken into account considerably by the recent stocking programs. The goal of our research was to assesse the genetic patterns, and relationships of the recent wild population and captured stocks.

Materials and methods

For the analysis to detect, we assessed the genotype of individuals with 12 microsatellites (DNA) markers from a wild population. The examined wild fish originated from Hungary parts of river Tisza and Danube, from *ex-situ* gene bank and two fish farms. The fish of the gene bank was marked with individual tags, this way we could collect genetic data on the individual level. For the analysis, we took samples from the tissue on the caudal fin. DNA isolation was conducted using the E.N.Z.A. kit tissue DNA (Omega Bio-tek Inc.) method, following the instructions set by the producer. We used 100 ng DNA per sample for the PCR reaction and 12 microsatellite markers for the genetic analysis into six PCR multiplex sets. Composition of PCR reaction based on the protocol of producer (Multiplex PCR Plus Kit, Qiage). The amplified products were determined using ABI 3130 sequencing (Applied biosystems, USA). The allele number and size were identified by the Gene Mapper (Applied Biosystem) software. The standard population genetic calculations were delivered by the GenALEx 6.5 statistical software package, and the discriminant analysis by R-program.

Results and discussion

As a result of the genotyping, we detected 126 alleles on the 12 loci. The population genetic values were calculated including the number of alleles, the number of effective alleles, the observed and expected heterozygosity, the fixation index (Table 1). The average allele numbers for the group varied between 5.33 and 9.8. The average values of fixation index per population were close to zero in all populations, in the Tisza population this value was even negative. The average expected heterozygosity (He) were between 0.52 and 0.59, while the observed (Ho) values were between 0.47 and 0.60. However, the differences were small, statistically significant differences were observed in the case of the most alleles. The gene bank group reached, the highest genetic diversity, we conclude it likely represents the population of the Middle Danube and its tributary Tisza river.

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Table 1. Data of the genetic diversity of the five examined groups

	N	Na	Ne	Ho	He	F
Genbank	139	9.08	4.47	0.55	0.56	0.01
Danube	47	8.00	4.69	0.54	0.59	0.09
Tisza	22	5.83	3.39	0.60	0.52	-0.16
1.FishFarm	30	5.33	3.63	0.47	0.57	0.13
2.FishFarm	30	5.83	3.03	0.49	0.53	0.01

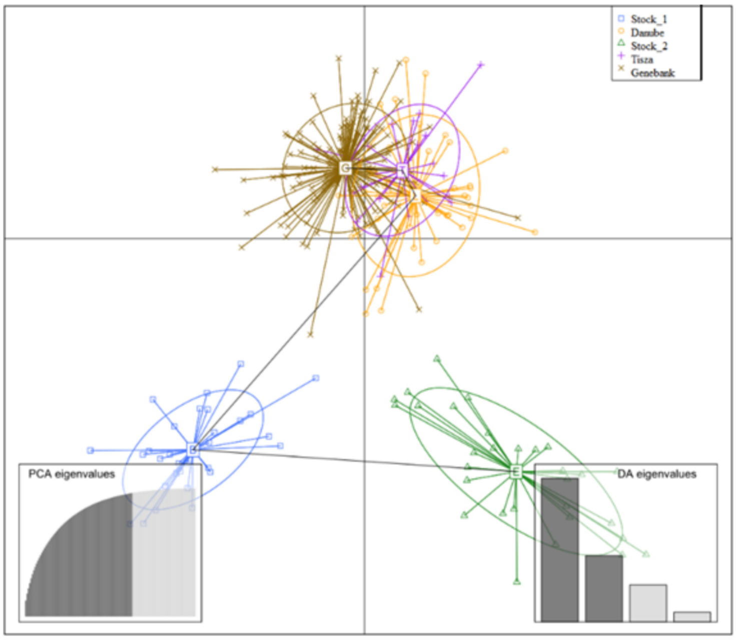

Figure 1. Principal component analysis (PCA) of microsatellite genotyping for all individuals. Brown data points gene bank, purple data points Tisza, orange datapoints Danube, blue data points Stock 1, green points Stock 2

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THE IMPACTS OF TITANIUM DIOXIDE NANOPARTICLES IN THE GONADS OF PACIFIC OYSTER: OXIDATIVE STRESS PROFILE AND ENERGY RELATED-PARAMETERS

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Introduction

The Pacific oyster (*Magallana gigas*) is the most produced oyster species worldwide, with France leading the European Union producers (86% of total production) (FAO, 2022). Due to its economic value, ecological role (e.g. filter feeders, reef-builders) and worldwide distribution, it is critical to assess the risk of emerging contaminants in this species. Titanium dioxide nanoparticles (TiO_2 NPS) appear as the most relevant NPs as regards human and environmental exposures, and their reprotoxicity is still scarcely addressed in oysters. Improving knowledge of oysters' reproductive biology, namely gonadal energy-related parameters and susceptibility to oxidative stress, is vital for population maintenance, stocking and predicting possible effects on other bivalve species.

Materials and Methods

The present study evaluated the effects of TiO_2 NPs on the oxidative stress profile and energy-related parameters of oysters' gonads. To accomplish that, juveniles (n=36) were exposed via water to environmentally relevant concentrations of TiO_2 NPs (10 and 100 µg/L of TiO_2 NPs) for 3 and 7 days. After exposure, the sex of the gonads was identified, and the antioxidant response (CAT, GPx, SOD, GR, GST and GSHt), lipid damage (LPO), the energy available (total carbohydrates, proteins and lipids content) and energy consumed (Electron transport system (ETS) activity) were assessed in female and male gonads.

Results

In male gonads, no alteration of the antioxidant responses and LPO occurred on day 3, while after 7 days of exposure, the activity of GPx increased in the 10 µg/L concentration concerning control, as well as the levels of LPO in both TiO_2 NPs concentrations. On the contrary, in female gonads, the activity of CAT decreased in the 10µg/L concentration and SOD in the 100µg/L relative to control, but no LPO appeared. After 7 days of exposure, no alteration occurred in the antioxidant responses and damage. Regarding the energy-related parameters of males, increasing lipid content at 100µg/L concentration and decreasing ETS at 10µg/L concentration occurred after 3 days of exposure, while after 7 days total carbohydrates content diminished in the 100µg/L concentration of TiO_2 NPs. Contrary to males, no alterations emerged for females after 7 days of exposure. However, decreasing carbohydrate content occurred at 100µg/L of TiO_2 NPs.

Discussion

Male gonads seemed to be protected against oxidative stress after 3 days of exposure to TiO2 NPs, while ETS activity decreased, suggesting impairment of its metabolic activity. Plus, lipid content increased in high TiO2 NPs concentration, which can alter membrane constitution and carbohydrate metabolism, a crucial energy source for gametogenesis. After 7 days of exposure, oxidative stress occurred in male gonads (LPO increased in both TiO_2 NPs concentrations), despite the significant increase in protection against oxidative damage (GPx increase) at lower TiO_2 NPs concentrations. Simultaneously to the enhancement of GPx and LPO, carbohydrates decreased, suggesting gonadal investment in detoxification mechanisms and less energy available for spermatogenesis. These findings suggest that male gonads are impacted differently, depending on the time of exposure. While after 3 days of exposure, the metabolic pathways were affected, after 7 days oxidative stress toxicity was also evident. Female gonads showed depletion of the antioxidant after 3 days of exposure (CAT and SOD), but with no oxidative stress, as no damage occurred. Similarly, the total content of carbohydrates also decreased, reinforcing gonadal investment in detoxification processes and less energy source for oogenesis. On the contrary, after 7 days of exposure, no alterations emerged in gonads. Apparently, females seem less vulnerable to TiO_2 NPs and show recovery ability after 7 days of exposure.

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Conclusion

The present results suggest that males and females have a different vulnerability to environmentally relevant concentrations of TiO_2 NPs and that females can cope with the challenge induced by these NPs. The effects of TiO_2 NPs in males may be enhanced with the exposure time, impairing gametogenesis. Further studies should evaluate the possible impacts of TiO_2 NPs in gonads of M. *gigas*, seeking to understand the potential mechanisms that interfere with reproduction, continuity of the species and impairment of production.

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GOODFISH: BUILDING BRIDGES BETWEEN PRODUCTION AND CONSUMPTION OF CULTIVATED FISH

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Introduction

Growth of the aquaculture sector in Europe and North America is a priority for food security, particularly as the world population increases and production from Asia and elsewhere becomes more accessible to domestic markets in those regions. The strategic aim of growing aquatic production in the West is sharpened by the policy goal of increasing per capita fish consumption, widely seen as key to dietary improvement, obesity reduction, and improved health and well-being.

The anecdotal distinction of quality between farmed and wild-caught fish is at least partly based on fallacious arguments and should not be the basis for consumer choice; however, there is a deficit of consumer information—at fresh fish counters, labelling typically provides the common name for a product, sometimes the scientific name, and origin. In the European Union, the provenance of wild-caught fish is reported only as a broad region (e.g. NE Atlantic), while cultivated products refer the country of origin.

This paradigm must shift towards significantly better consumer information, presented in an appealing way, that helps buyers understand what they purchase and why—in essence, what defines a good fish.

Concept and approach

A good fish can be defined through the following four categories, each with a corresponding set of descriptors or indicators:

1. Consumer experience (taste, texture, etc);
2. Nutrition and well-being (composition, including key factors such as Omega-3 or calcium content, and health benefits, such as neo-natal cerebral development or anti-inflammatory properties);
3. Environmental sustainability (e.g. associated to ecological carrying capacity, see Ferreira et al., 2012);
4. Animal welfare (metrics on environmental thresholds, stocking and harvesting, etc).

This classification does not distinguish between aquaculture and capture fisheries, but instead uses an evidence-based categorical approach in the definition of what constitutes a good fish. The implementation of the GoodFish methodology required (a) a well-established scientific basis for extracting and providing relevant information; and (b) a simple and effective means of communication of this information to the consumer, using an actionable, two-way approach. These requirements will be reviewed in turn.

Sourcing and processing of information

Organoleptic qualities were sourced from hospitality industry databases and interviews with chefs, and included ancillary data (e.g. product suitability for preparation of specialty presentations such as sushi or ceviche). The composition of cultivated aquatic products was analysed by means of well-established food composition databases from both the US Food and Drug Administration (USA) and Public Health England (UK). From these sources, a cloud-based relational database was developed containing detailed data on proximate composition, inorganics, vitamins, saturated and unsaturated fatty acids, and a number of other constituents.

Human health information was collected from the literature, as was data on environmental thresholds and animal welfare conditions (see e.g. https://meta.longline.co.uk/, which compiles data on 500 thresholds sourced from about a thousand scientific publications).

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Communication and feedback

A smartphone application was developed, accessible via a QR code integrated in the labelling of fish and shellfish sold fresh at supermarkets and fishmongers or prepared in specialty restaurants. The GoodFish platform differs from other offerings—it does not present a score or ranking of particular products i.e. it does not make recommendations or attempt to make decisions on behalf of the consumer. Instead, the emphasis is on displaying information in a clear, simple, and appealing way, allowing consumers to make up their own mind, and providing a means for three-stage feedback on products. This feedback uses the ACT[1] metaphor: Acquire – Cook – Taste. After scanning a product QR code or set of codes, consumers are invited to classify their purchasing experience using the usual five-star scheme. Subsequently, the consumer can score the product in terms of ease and enjoyment of preparation (confection), and finally with respect to the experience of eating the resulting dish.

The app is supported by a web-based platform (http://goodfi.sh) that operates the databases and allows consumers and businesses to manage various options. For supermarkets and restaurants, this includes product management, access to scores, and other business intelligence data.

The deployment and refinement of the GoodFish app is currently being discussed with a major retail group, with the aims of providing consumers with a much richer experience in their relationship with aquatic foods, increasing understanding, and stimulating *per capita* consumption. Retail outlets currently have little grasp of the actual consumer experience—once a fish fillet or a bag of mussels is sold, there are no mechanisms to provide feedback on the way a product was prepared and whether the resulting meal was forgettable, enjoyable, or delicious. As an increasing number of consumers provide their own ACT scores for aquatic products, the resulting information can be leveraged to understand patterns of consumption, species preferences, and relevance of factors such as traceability and local sourcing, and help aquaculture producers tailor their offerings to better meet consumer expectations.

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In Latin languages, this becomes the 3C approach, e.g. in Italian: *Comprare – Cucinare – Consumare*.

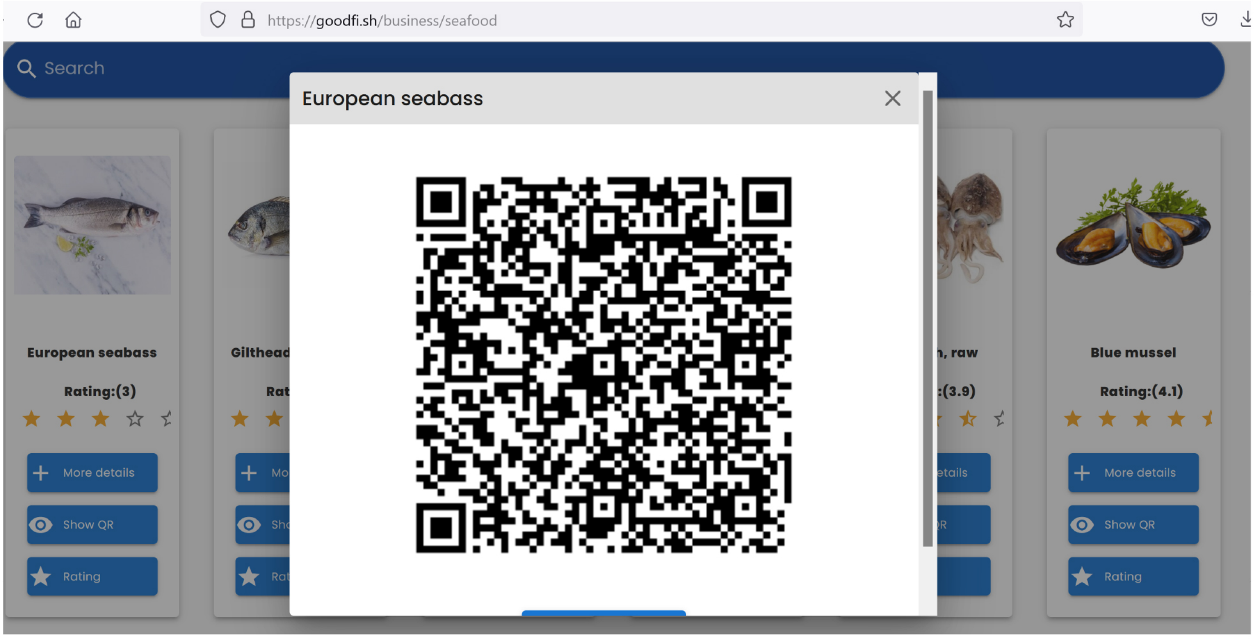

Fig. 1. GoodFish business intelligence platform showing the QR code for European seabass. Scan the code on your smartphone.

INTEGRATED CARRYING CAPACITY MODELLING FOR FINFISH CULTURE IN LAKE VICTORIA

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Introduction

The population of Sub-Saharan Africa is growing at an estimated 2.7% per year, more than twice that of South Asia and Latin America. This growth rate will lead to a doubling of the region's population by 2050, which poses a significant challenge with respect to food security.

Capture fisheries in the whole of Africa are well above aquaculture production, which contrasts sharply with the global situation (FAO, 2020)—since 2013, over half of the seafood for direct human consumption is sourced from aquaculture. Contrary to world fisheries, which are flatlining, African wild catch is still increasing, but the share of aquaculture in production of seafood has tripled since the year 2000. In parallel, *per capita* fish consumption in sub-Saharan Africa is very low, generally below 5 kg ind^{-1} y^{-1}. Out of the three nations bordering Lake Victoria, only Uganda consumes over 10 (10-20) kg ind^{-1} y^{-1} (FAO, 2020).

The decline of capture fisheries in the Great Lakes due to overfishing and other factors has been ongoing for several decades (Ogutu-Ohwayo et al, 1997), and catches of Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) in Lake Victoria have decreased markedly since the 1990s (Katunzi, 2019).

The dual challenges of collapse of capture fisheries and population growth—the population of the Kenyan catchment of Lake Victoria is expected to double by 2050, in line with the rest of Sub-Saharan Africa—mean that aquaculture is one of the potential solutions to allow expanding human populations to have access to food security over the next thirty years and beyond.

Tilapia, due to its high growth rate, well-established husbandry, and low trophic status, shows great promise as a high-quality protein source, and Lake Victoria has appropriate conditions for its cultivation. However, in order to ensure that aquaculture expansion in this region is achieved in a sustainable manner, a development framework is required to address the challenges of environmental sustainability and ecosystem carrying capacity.

In order to illustrate the underlying issues, and in particular the challenges in the implementation of the Ecosystem Approach to Aquaculture (EAA - Soto et al, 2008), taking into account ecological sustainability, social balance, and harmonization of multiple uses, a well-tested framework for zoning and carrying capacity assessment is being applied in Lake Victoria. The rationale is that models of this kind can be used to support industry development and help managers make informed decisions about sustainable expansion of aquaculture.

Approach

The framework used for carrying capacity assessment builds on a combination of various models, which together are used to understand the different components of the system (*Fig. 3)*. These include individual growth models, local-scale carrying capacity models, farm footprint models for both organic and inorganic waste, models for land use in order to partition nutrient loading, and spatial analysis tools.

This complex framework is being developed as part of the Sustainable Activities in Water Areas (SAWA) project, developed by Kenya, which aims to build an aquaculture governance framework that improves decision-making and addresses the challenges of multiple lake uses in the Kenyan part of Lake Victoria.

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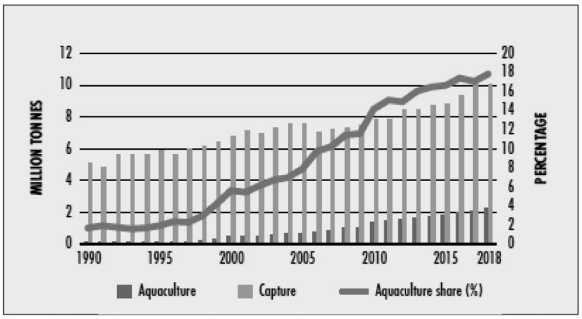

Fig. 1. Wild capture & aquaculture in Africa (FAO, 2020).

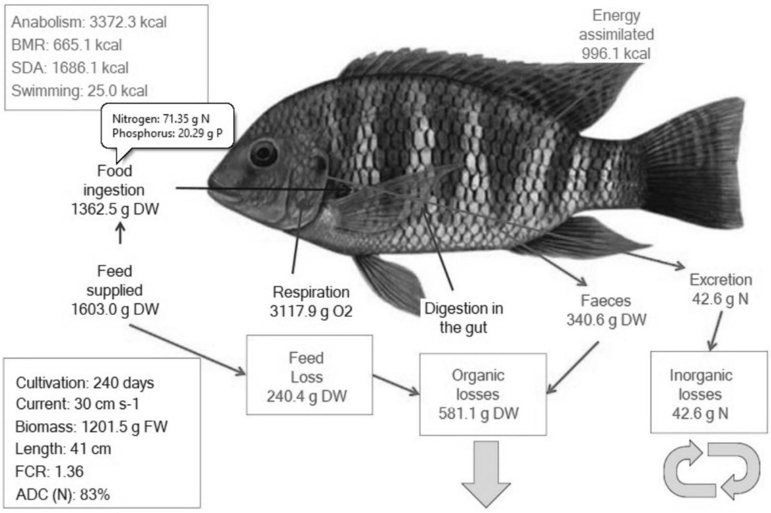

Fig. 2. Individual model for Nile tilapia. The AquaFish models are used to drive local-scale carrying capacity models such as FARM.

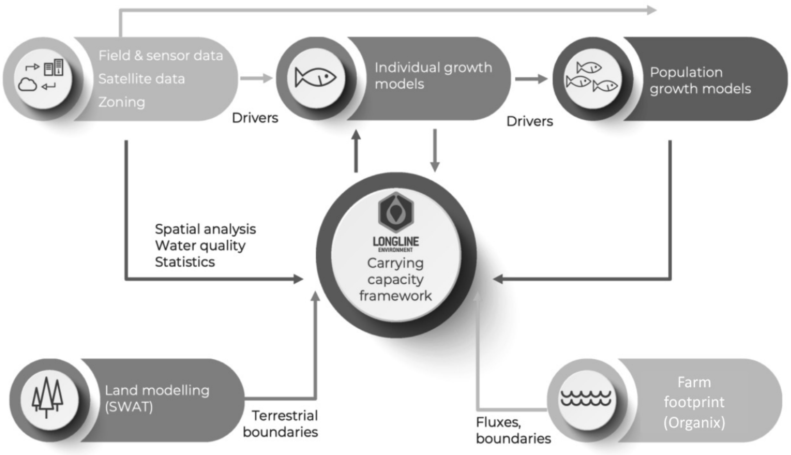

Fig. 3 –General modelling framework, showing the different types of models and respective interactions.

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In parallel to the modelling framework, a full stakeholder consultation and engagement programme is developed within SAWA. The results obtained from the natural sciences components of the work will help to inform factually-based discussions and promote consensus among aquaculture businesses, fishers, management agencies, and the wider community.

The application of the EAA for the Kenyan area of Lake Victoria will create conditions for industry growth, contemplating both artisanal cultivation practiced by local communities associated to Beach Management Units (BMU) and more sophisticated operations that include full vertical integration from farm to fork.

Results and discussion

A mass balance for individual growth modelling for Tilapia, using the well-tested AquaFish model (e.g. Ferreira et al, 2015) is shown in Fig. 2. The model will be tuned for Lake Victoria, where production of market-sized tilapia takes longer than in Thailand or China, which may be related to feed quality or to other husbandry aspects. The model forms the basis for local-scale modelling of tilapia farms, which in turn will be used to drive simulations of organic deposition and enable the assessment of ecological carrying capacity.

Results for organic loading to the sediment from commercial tilapia farms using the ORGANIX model suggest that at present, finfish aquaculture developed sustainably has significant upside for increasing food security and meeting targets for consumption of aquatic products in Kenya.

Fieldwork and stakeholder consultation showed that both local communities and larger businesses are committed to work within SAWA to provide data and improve the knowledge base essential for the successful application of EAA to Lake Victoria, and fully expect to benefit from the project outcomes. A frequent stakeholder concern was to ensure that the results of SAWA have the necessary buy-in from policy makers as a condition for success.

We hope that the implementation of a successful aquaculture framework for the Kenyan water area, taking into account the uses of the associated catchment, will exemplify how this participative governance model can be extended to Lake Victoria as a whole, a transboundary system that must be managed holistically.

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ASSESSING THE POTENTIAL FOR NUTRIENT TOXICITY ON SEAGRASS IN THE VICINITY OF AN AQUACULTURE SITE

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Introduction

Finfish aquaculture is a source of dissolved nutrients which can affect water quality in the wider environment (Howarth et al. 2019). Therefore, the potential effects of dissolved nutrient loading must be considered if management is to transition towards an Ecosystem Approach to Aquaculture (EAA). Understanding the extent and effects of dissolved nutrient plumes emanating from fish farms has been identified as one of the largest knowledge gaps in transitioning towards the EAA (Jansen et al. 2018). In this study, the dispersion of dissolved nitrogen from a rainbow trout farm in Port Mouton (Nova Scotia, Canada) was investigated using a fully spatial hydrodynamic model. The ecosystem level effects were evaluated as the potential for dissolved nitrogen toxicity on a foundation seagrass species, particularly given that seagrass has declined in Port Mouton over time.

Material and Methods

Dissolved nitrogen loading was calculated by applying nutritional mass balance model to growth estimates from a Thermal Growth Coefficient model. Both models were parameterized for the observed conditions in Port Mouton. The spatio-temporal dynamics of dissolved nitrogen was simulated by coupling a tracer sub-model to a hydrodynamic model constructed in FVCOM (Finite Volume Community Ocean Model). The tracer submodel was restricted to advection-dispersion dynamics, omitting any chemical and biological processes. These conservative tracer simulations maximized nitrogen levels, and consequently, were considered a worst-case scenario to inform ecosystem level effects under a precautionary approach. A range of scenarios were applied for different stocking densities, background conditions and aquaculture practices.

Results and Discussion

The aquaculture scenarios simulated in Port Mouton suggest that the maximum concentration of dissolved nitrogen would not exceed 6 μM in the center of the farm under the most adverse conditions (2 μM background nitrogen, 500,000 fish, peak of production, and no uptake of nutrients by primary producers). This concentration would drop to < 5 μM for the most common scenario taking into account the aquaculture practices at the farm (Fig. 1). These values are below the toxicity threshold for seagrass reported in the literature. To the best of our knowledge, the lowest value tested in the literature for ammonium toxicity was 9 μM (van Katwijk et al. 1997). These authors reported that toxicity for *Zostera marina* occurs at ammonium concentrations between 9 and 25 μM, with no effects at 9 μM and evident necrosis at 25μM.

Models are simplifications of the real-world and this is not an exception; however, the assumptions to simplify the model were directed to simulate the worst-case scenario and would maximize the buildup of dissolved nutrients, and consequently, toxicity for seagrass. First, the model did not distinguish between chemical forms of nitrogen, which represents the worst-case scenario to define toxicity for seagrass, given that the toxicity is specific to each nitrogenous compound (e.g. van Katwijk et al. 1997). Second, only background concentration of dissolved nutrients and the contribution of the farm were used as sources of nitrogen, and more importantly, no sinks were included in the model in the most stringent scenarios. This approach implies that dissolved nitrogen can only leave the system through the exchange with the open ocean, which increases the residence time of dissolved nitrogen in the system, increasing potential for toxicity. Third, background concentration of dissolved nitrogen exceeded observed values in adjacent waters (Johnson et al. 2018), which accounts for uncertainty. Fourth, the biomass of fish was overestimated in some scenarios, and it was simulated during peak of production, which represents the worst-case scenario in terms of excretion of nitrogenous products. These four considerations ensure a precautionary approach when evaluating the model's predictions for dissolved nitrogen in the context of potential toxicity for seagrass.

The simulation of dissolved nitrogen as a conservative tracer using a fully-spatial hydrodynamic model suggests that the maximum concentration of nitrogen caused by a trout farm is unlikely to be toxic for seagrass in Port Mouton Bay. This conclusion is grounded in the analysis of precautionary worst-case scenarios that aim to maximize the buildup of dissolved nitrogen in the bay. This outcome suggests that the decline of seagrass reported in some parts of the bay are unlikely to have been triggered by dissolved nutrients discharged from the farm.

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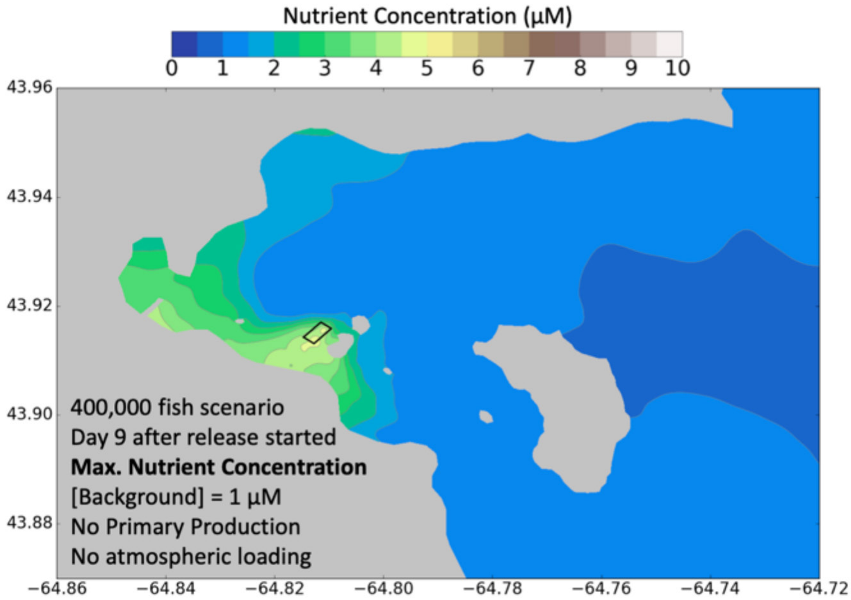

Fig. 1. Maximum predicted nutrient concentration (µM) in the 400,000 fish scenario after 9 days using as a background nutrient concentration of 1µM, no primary production, and no atmospheric loading.

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IMPACT OF SUSTAINABLE MARINE LIPID SOURCES ON MUSCLE QUALITY OF FARMED EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

Fish is an important source of macronutrients, vitamins and minerals, being our primary source of omega-3 fatty acids, namely EPA and DHA. This characteristic associates the ingestion of fish with cardiovascular, intestinal, and neural benefits, with aquaculture playing an essential role since it contributes to nearly half of the global fish consumption. However, the sector´s sustainability raises questions, leading the aquaculture industry to reduce the inclusion of fish oil (FO) in fish feed, and replace them with other more sustainable oils like soybean oil (SO) but devoid of omega-3 fatty acids (Kriton et al., 2018). Here, one of the biggest challenges is to find more sustainable ingredients that equally provide the demands of omega-3 to the final consumer and recommend by WHO. Another challenge is to maintain the organoleptic characteristics of the fillet since the increase in fatty acids content can lead to a more perishable product. Therefore, the aim of this study was to address if the substitution of FO and SO with sustainable marine sources rich in EPA and DHA – salmon oil (a coproduct from the salmon industry), algae oil and algae blend - in sea bass feeding could result in quality alterations during a 14 day storage period in ice.

Materials and methods

European sea bass (*Dicentrarchus labrax*) (initial body weight 71.1 ± 13.2 g) were fed for 116 days with two experimental diets (Marine 1 and Marine 2) and compared to counterparts that received a commercial-like diet (control). All diets were isoproteic (45% dry matter, DM) and isolipidic (18% DM) with different lipid sources (Table 1): Control diet is a commercial-based diet with a blend of sardine and soybean oil as primary lipid sources; Marine diets 1 and 2 were completely devoid of SO: in diet 1, FO was partially replaced by salmon oil (a coproduct from the salmon industry) and a blend of micro and macroalgae; in diet 2, FO was totally replaced by a blend of salmon and algae oil. All the diets were tested in quadruplicate. By the end of the trial, all fish were individually weighed and 4 fish per tank were sampled for flesh quality evaluation over a 14 day storage period in ice. Fish fillets were evaluated for colour, pH, water holding capacity (WHC) and texture at days 0, 3, 7 and 14. Previous reports indicated 14 days as the sea bass shelf life, so this was selected as the end point of the experiment (Kılınc et al., 2007; Özden et al., 2007). Muscle samples were also collected for antioxidant activity and lipid oxidation at days 0, 3, 7 and 14 (ongoing).

Results

In the present study, sea bass fed with diets in which FO and SO was partially (Marine 1) or totally replaced (Marine 2) by alternative marine sources, grew equally well as fish fed with the control diet. No significant differences could be observed in fish survival, growth performance, and feed efficiency.

The evolution of pH throughout time is presented in Figure 1a. For all diets, an increase in pH during storage was observed, which is in agreement with other previous studies that reported a rise in pH associated with volatile bases production due to the degradation of protein to ammonia and trimethylamine by microbial or endogenous enzymes (Monirul et al., 2019). The maximum value for pH was at day 14 with 6.65, 6.62 and 6.60 for control, Marine 2 and Marine 1 diet, respectively. The experimental diets did not significantly affect flesh pH. Also, no differences were observed for either water holding capacity (WHC) (Figure 1b) and colour among fillets from fish fed with the various experimental diets.

Regarding the textural properties, no significant differences were observed for hardness between the control and the experimental diets. The average values for springiness and resilience displayed significant changes at day 7 with sea bass fed with Marine 2 diet, which exhibited the highest values. In addition, fish fed with Marine 2 diet presented significantly higher values for cohesiveness in comparison to the control diet at the end of storage (day 14).

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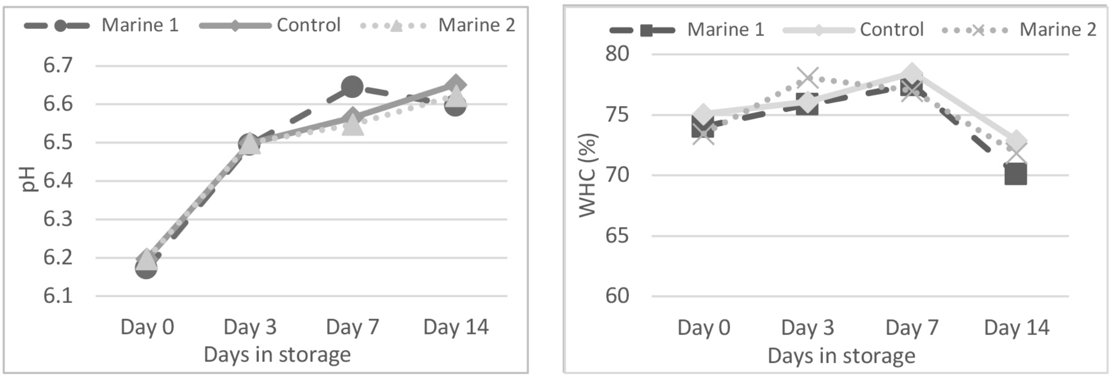
Table 1. Lipid sources and EPA+DHA content of the experimental diets.

Ingredients (%)	Control	Marine 1	Marine 2
Fish oil	9.0	6.7	-
Soybean oil	4.4	-	-
Salmon oil	-	6.2	10.1
Algae oil	-	-	3.3
Blend of algae	-	2.0	-
Total dietary EPA+DHA (%)	3	3	3

Figure 1. pH and water holding capacity (WHC) of the sea bass fillet over the 14 days storage period.



a)

b)

Discussion and Conclusion

The fact that the type of feed had no negative effect on the muscle textural parameters studied suggests that marine lipid sources rich in omega-3 could be an excellent sustainable alternative to FO in aquafeeds, whilst assuring fish nutritional quality and consumers' health. Moreover, sea bass fed with salmon oil combined with algae oil improved flesh cohesiveness, suggesting an improvement in fish muscle integrity in comparison with sea bass fed with control diet, and therefore, the potential to facilitate the shelf life of sea bass fillets during 14 days of storage at refrigerated temperatures.

Acknowledgements

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EFFECT OF SOLID-STATE FERMENTATION EXTRACTS OBTAINED WITH WINERY AND OLIVE MILL BY-PRODUCTS ON OXIDATIVE STRESS REDUCTION IN EUROPEAN SEA BASS DIETS

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Introduction

With the ban on ethoxyquin and concerns about the use BHT and BHA in the European Union, novel alternatives of antioxidants for aquaculture have become an issue. The most promising replacements for these synthetic antioxidants are natural antioxidants primarily present in plants. A source of high amounts of natural antioxidants can be found within by-products from the olive-mill and winery industries, with are prominent in the EU. However, antioxidants bioavailability may be compromised due to the lignocellulosic matrix of these by-products. Solid-state fermentation (SSF) is a sustainable bioprocess that can utilize agro-industrial by-products as a substrate for microbial growth in low water activity conditions, producing enzymes that hydrolyze the cell wall and release antioxidants into the substrate. After SSF, these important bioactive compounds are recovered with different applications, including feed additives for aquaculture. Dietary fortification with antioxidants can be beneficial in reducing fish's oxidative stress status and delaying lipid peroxidation. This work aimed to study the antioxidant potential of extracts from olive mill and winery by-products, unfermented or fermented, in European Sea Bass.

Materials and methods

An optimized mixture of olive mill and winery wastes (Filipe et al., 2019) was fermented by SSF with *Aspergillus ibericus* (MUM-01.29; Micoteca od the University of Minho (UM). After fermentation, an aqueous extraction was performed (1:5 solid/water, w/v) for 30 minutes with constant stirring. Another extract was also prepared from the same mixture but without the fermentation process. The recovered extracts were then lyophilized (FWO and UWO, fermented and unfermented extracts, respectively). Four isolipidic and isoproteic diets (18% lipids, 50% protein) were formulated with 0 (control diet), 0.34, and 0.68% of FWO or with 0.19% of UWO to reach an antioxidant activity of 0 (control diet), 683 (FWO1 diet), 1365 (FWO2 diet) and 683 (UWO diet) μmol Trolox kg^{-1} of diet. A growth trial with European sea bass (IBW= 21.5g) testing each experimental diet in triplicate was performed for 66 days. At the end, the liver and intestine of 9 fish from each diet were sampled, immediately frozen in liquid nitrogen, and stored at -80º C. Samples were then homogenized in Tris–HCl buffer at a pH of 7.8, centrifuged at 30,000×g for 30 min at 4 °C and supernatants were recovered. Superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and thiobarbituric acid reactive substances were analyzed in both tissues.

Results and discussion:

In the liver (Table 1), there is a general decrease in specific oxidative stress-related enzyme activity without affecting liver peroxidation with the dietary supplementation with FWO or UWO extracts. Antioxidant compounds present in these diets may have had a direct action on preventing or neutralizing the reactive oxygen species (ROS) production, modulating the activation of oxidative stress enzymes.

In the intestine (Table 2), a general increase in specific oxidative stress-related enzyme activity, together with a decrease in lipid peroxidation, was observed with the dietary inclusion of FWO1 or UWO extract. Besides their direct ROS scavenging activity, dietary inclusion of FWO1 and UWO extracts also activated the antioxidant enzymes system. Compared to the FWO1 diet, the FWO2 diet induced lower activity of the antioxidant enzymes and increased intestinal lipid peroxidation.

Conclusion

Present results showed that dietary inclusion of FWO and UWO extracts at a level corresponding to the antioxidant activity of 683 μmol Trolox kg^{-1} diet improved the intestine's antioxidant status, increasing its ability to cope with ROS production and decreasing the lipid peroxidation. In the liver, these extracts reduced the overall antioxidant enzymes activity without compromising the hepatic peroxidation

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Table 1. Oxidative stress enzymes (U mg protein^{-1}) and peroxidation (nmol MDA g^{-1} tissue) in the liver of fish fed experimental diets

Diatary DPPH (μmol Trolox kg^{-1} diet)		**SOD**	**Catalase**	**GPX**	**GR**	**G6PD**	**TBARS**
Control	0	70.8^{a}	479.5^{a}	35.0	7.91^{a}	350.1^{a}	12.7
FWO1	683	58.6^{b}	436.2^{ab}	35.4	7.20^{ab}	298.7^{ab}	11.3
FWO2	1365	61.9^{ab}	288.6^{b}	37.5	7.33^{ab}	270.5^{ab}	13.1
UWO	683	51.6^{b}	399.7^{ab}	31.7	5.70^{b}	246.6^{b}	12.3
SEM	-	1.91	25.6	1.78	0.30	14.06	0.31

FWO, fermented wine and olive extract; UWO, unfermented wine and olive extract; DPPH, total antioxidants; SOD, superoxide dismutase; GPX, glutathione peroxidase; GR, glutathione reductase; G6PD, glucose-6-phosphate dehydrogenase; TBARS, thiobarbituric acid reactive substances.

Values are presented as means (n=9) ± standard error of the mean (SEM). Significant differences between means are represented with lower case letters

Table 2. Oxidative stress enzymes (U mg protein^{-1}) and peroxidation (nmol MDA g^{-1} tissue) in the intestine of fish fed experimental diets

Diatary DPPH (μmol Trolox kg^{-1} diet)		**SOD**	**Catalase**	**GPX**	**GR**	**G6PD**	**TBARS**
Control	0	145.8	660.0	3.8	16.9	12.6	70.4^{a}
FWO1	683	181.9	933.7	8.4	22.2	13.2	40.4^{b}
FWO2	1365	145.1	719.9	5.2	18.7	10.6	66.9^{ab}
UWO	683	181.7	747.0	10.4	22.0	18.6	47.0^{ab}
SEM	-	9.61	44.4	0.67	1.04	0.95	4.14

FWO, fermented wine and olive extract; UWO, unfermented wine and olive extract; DPPH, total antioxidants; SOD, superoxide dismutase; GPX, glutathione peroxidase; GR, glutathione reductase; G6PD, glucose-6-phosphate dehydrogenase; TBARS, thiobarbituric acid reactive substances.

Values are presented as means (n=9) ± standard error of the mean (SEM). Significant differences between means are represented with lower case letters

EFFECT OF SOLID-STATE FERMENTATION EXTRACTS OBTAINED WITH WINERY AND OLIVE MILL BY-PRODUCTS ON GROWTH OF ZEBRAFISH (*Danio rerio*) UNDER PHYSIOLOGICAL STRESS

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Introduction

The use of dietary supplements in the form of antioxidants is already commonplace in aquaculture diets. Antioxidants are used to reduce aquafeed lipid peroxidation and to improve fish oxidative status. Furthermore, antioxidants are vital for protein turnover as they reduce oxidative damage in the skeletal muscle thus promoting long-term growth performance. As synthetic antioxidants like diphenylamine and ethoxyquin were banned in Europe due to food safety concerns, novel antioxidants made from natural sources are becoming predominantly researched. By-products from olive mills and wineries are important agroindustrial industries in the Mediterranean region and are a good source of natural antioxidants. However, the bioavailability of these antioxidants may be impaired by the lignocellulosic matrix of these by-products. Solid-state fermentation (SSF) is an economical and ecological bioprocess that can be applied to extract the highest amount of antioxidants from these by-products. SSF uses fungi to hydrolyze the aforementioned lignocellulosic matrix and release phenolic compounds, which become available to be recovered, concentrated, and added to the diets. This work aimed to evaluate how extracts obtained from fermented and unfermented wine and olive oil by-products affected the growth and feed utilization of fish maintained under non-stressful or chronic handling stressful conditions, using Zebrafish (*Danio rerio*) as a biological model.

Materials and methods

An optimized mixture of exhausted olive pomace, vine-shoot trimmings, and exhausted grape pomace for increased enzyme production and antioxidant recovery (Filipe et al., 2019) was fermented by SSF with *Aspergillus ibericus* (MUM-01.29; Micoteca of the University of Minho (UM). After fermentation, an aqueous extraction was performed (1:5 solid/water, w/v) for 30 minutes with constant stirring. Another aqueous extract was prepared from the same mixture but without the fermentation process. The extracts were lyophilized and the final products were labeled as FWO (fermented extract) and UWO (unfermented extract). Three isolipidic and isoproteic diets (8% lipids, 40% protein) were formulated: a control diet, without extracts, and two diets with fermented (FWO diet) and unfermented (UWO diet) extract included at 1 mg gallic acid equivalents per g of diet.

The trial was performed with zebrafish (*Danio rerio*) with an IBW of approximately 96 mg. Diets were tested in quadruplicate for 24 days under non-stressful conditions. Then, fish were weighed, and in the following 5 days, 2 of the 4 replicates were exposed to daily stress by air exposure for 3 minutes, while the other 2 replicates were not stressed. During this period, stressed and unstressed fish were fed the same diets. Then, fish were weighed, and weight gain, specific growth ratio, feed intake, and feed efficiency were determined.

Results and discussion:

During the non-stress period of the trial, corresponding to the first 24 days of the trial, no effect of dietary supplementation with FWO or UWO extract on growth performance and feed utilization efficiency was observed. During the last 5 days, corresponding to the stress period, relative to the control, the diet supplemented with the FWO extract led to significantly higher weight gain, feed efficiency, and specific growth ratio.

When evaluating the whole trial, from day 1 to day 29 (**table 1**), including unstress and stress periods, relative to the control, the diet supplemented with the FWO extract led to a significantly higher weight gain and specific growth ratio. Both diets supplemented with FWO extract or UWO extract led to a significantly lower feed intake and higher feed efficiency. Stress did not affect growth performance and feed utilization.

Conclusion

The FWO diet promoted growth performance and both the FWO and UWO increased feed utilization and decreased feed intake, relative to the control diet.

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Table 1- Effect of dietary supplementation with FWO and UWO on growth performance and feed utilization efficiency of zebrafish from day 1 to 29.

	Control		FWO		UWO		SEM
	NS	S	NS	S	NS	S	
Final Body weight (mg)	141.8	135.8	160.2	167.1	150.0	156.7	4.5
Weight gain (g/kg ABW^{-1} day^{-1})	20.1	20.9	31.3	26.9	24.6	26.5	1.3
Feed Intake (g/kg ABW^{-1} day^{-1})	66.1	72.9	61.1	59.7	60.6	56.0	1.8
Feed efficiency	0.3	0.3	0.5	0.5	0.4	0.5	0.0
Specific growth ratio	2.0	2.1	3.2	2.8	2.5	2.7	0.1

Two-way ANOVA	Diet	Stress	Interaction	Diets		
				control	FWO1	UWO1
Final Body weight (mg)	n.s	n.s	n.s			
Weight gain (g/kg ABW^{-1} day^{-1})	**	n.s	n.s	a	b	ab
Feed Intake (g/kg ABW^{-1} day^{-1})	***	n.s	n.s	b	a	a
Feed efficiency	***	n.s	n.s	a	b	b
Specific growth ratio	**	n.s	n.s	a	b	ab

n.s - non-significant; ($P \geq 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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IN-DEPTH COMPARATIVE MICROBIOME ANALYSIS OF MEDITERRANEAN FISH HATCHERIES

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Introduction

The importance of the fish hatcheries' microbial communities to fish health and welfare has been long recognized and several studies have been conducted to map these communities during healthy rearing conditions but also during disease outbreaks. Most studies focus on the intestinal and epidermal microbiome of the developing fish, which is mainly shaped by the live feeds (microalgae, rotifers and copepods) used at the rearing process. The microbiome of live feeds, however, has not been investigated thoroughly so far.

Objective

We have analyzed the microbiome of one research and two commercial fish hatcheries producing Mediterranean fish species with emphasis on their live feed sectors. The aim was to compare the microbiomes of hatcheries with different characteristics (water source, production intensity and practices) and provide baseline information about their structure with focus on putative pathogenic bacteria.

Materials and Methods

The hatcheries were sampled seasonally over one year. Sampling involved 5 stations: inlet, microalgae, rotifers, artemia and outlet. Water samples were filtered using a vacuum filtration system and DNA was extracted from filters. 16s amplicon Novaseq sequencing was conducted. Bioinformatics included amplicon sequence variant analysis (ASVs).

Results

More than 162.859 good quality sequences per sample were generated in a total of 129 samples. 46.745 ASVs were annotated with 362 average ASVs per sample. Opportunistic fish pathogens like *Vibrio alginolyticus* and *Vibrio anguillarum* are abundant in the Artemia sections of the commercial hatcheries. The genera of *Vibrio* and *Tenacibaculum* are abundant and diverse in all hatcheries with the incoming water contributing significantly to the presence of important taxa that constitute presumptive pathogens. Alpha diversity analysis revealed a relatively stable microbiome between seasonal samplings for every hatchery. Nevertheless, a diverse microbiome between sampling stations was discovered. A core microbiome for every hatchery was catalogued, which included important putative fish pathogens belonging to *Vibrio* and *Tenacibaculum* genera. Mapping of the microbiome in the live feeds through metagenomics is the first step towards an efficient microbial control in the fish hatcheries.

Acknowledgements

This work received funds from the Operational Program Fisheries and Maritime 2014–2020 in the framework of the project "Biological control of pathogens in fish hatcheries using bacteriophages" 5010932.

BIOSTIMULANT PROPERTIES OF *Euglena gracilis* BIOMASS GROWN ON PRE-TREATED EXHAUSTED DAIRY BYPRODUCTS

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Introduction

Some byproducts of the dairy industry, such as the exhausted whey from Bovine Ricotta cheese production (scotta), do not have real ways of valorization as the bovine whole whey but, being still rich in nutrients, need to be disposed of for not impacting on the environment. Current conventional treatment technologiesare of hard management and feature high costs. Among the possible and more sustainable solutions, microalgae-based bioprocesses could be a valid alternative (Gramegna et al., 2020). *Euglena. gracilis* is a microalga able to grow under heterotrophic and mixotrophic conditions, accumulating higher amounts of paramylon than under photoautotrophic conditions (Lewis et al., 2020). Paramylon can be used in many fields of commercial interest. Some studies also indicated biostimulant properties of this polysaccharide (Barsanti et al., 2019).

In this work, the ability of *E. gracilis* to grow on diluted ricotta exhausted whey was verified. The obtained biomass was than characterized for protein, paramylon and lipid content and used for evaluation of its biostimulant effects.

Materials and methods

A sample of ricotta whey, coming from an Italian dairy, underwent a preliminary treatment consisting in thermocalcic precipitation (Bosco et al.,2018), in order to remove the lipid fraction and obtain a clarified phase. This phase had a significantly lower COD (from 55 g L^{-1} to 35 g L^{-1}). A part of the clarified phase was then, in turn, subjected to microfiltration, in order to reduce its bacterial count. Three growth media were tested with *E. gracilis* (1224-5/25): i) Standard Cramer-Myers medium (SM, Cramer & Myers, 1952) with glucose, used as control (SMG); ii) clarified whey, diluted 1:3 with water (TCW1); iii) microfiltered clarified whey diluted 1:3 with water (TCW2). Growth tests were performed in 100-L bubble-column photobioreactors; each medium was tested in duplicate in batch mode, at a temperature of 20-25° C and an irradiance of 80 μmol photons m^{-2} s^{-1}, with a dark/light cycle of 12/12 hours. At the end of the growth-phase, the biomass was harvested, analyzed for biochemical parameters and tested to assess seed germination on cress (Lepidium sativum L.) by a phytotoxicity test (Ronga et al., 2019).

Results

The best growth was found in TCW2, where the maximum cell density at day 10 was 0,88 ± **0,04** Mcell mL^{-1} and COD was removed by about 75%. The inorganic nitrogen was removed by 90-95% in both TCW1 and TCW2 and fully depleted in the control (SMG). The culture tests of *E.gracilis* on exhausted whey showed a content of proteins almost 2-fold higher than the control (SMG: 25.4 ± 0.6%; TCW1: 49.8 ± 8.4%; TCW2: 49.5 ± 3.4%). On the contrary, the content of paramylon (% of dry matter) was 2-time higher in the control compared with the tests (SMG: 21.9 ± 0.9%; TCW1: 9.7 ± 1.4%; TCW2: 12.0 ± **2.1%). The results of phytotoxicity test revealed an interesting** biostimulant effect on cress seeds of the TCW2 extract, with an average germination index of 150.9 ± 17.6% (with respect of water, control thesis), while the extracts of the SMG (108.9 ± 9.2%) and TCW1 (96.8 ± 2.0%) revealed neither biostimulant nor phytotoxic effects.

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Discussion and Conclusion

This study showed the ability of *E. gracilis* to grow on a pre-treated and water-diluted exhausted whey without additional nutrient supplementation.

The biochemical composition of the *E. gracilis* biomass grown on the dairy byproduct was found to be significantly different from the one grown on synthetic medium. In particular, the microalgae biomass grown on dairy byproduct, thus with lactose as source of carbohydrate and whey proteins and peptides as additional organic and nitrogen sources, showed a higher protein content, at the expense of paramylon and lipids. On the contrary, the *E. gracilis* biomass grown on synthetic medium, thus with glucose as the only organic source, showed a low content of protein and higher content in lipids and paramylon. The only microalgae biomass showing a biostimulants effects was that grown on microfiltered exhausted whey (TCW2).

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ELEMENTAL FINGERPRINTING OF WILD AND FARMED GILTHEAD SEABREAM (*Sparus aurata*) MUSCLE TOWARDS PRODUCT AUTHENTICATION AND VALUATION

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In the context of expanding fish production and complex distribution chains, food safety assessments and the development of traceability tools become increasingly important. Here, the elemental fingerprints of gilthead seabream (*Sparus aurata*) muscle from four sources in the Northeast Atlantic were assessed, namely from two Portuguese locations, representing three production methods: from Olhão (Mainland Portugal) captured in i) wild, ii) semi-intensive aquaculture in earth ponds, and iii) intensive sea cage aquaculture; and from Madeira Island captured in iv) intensive sea cage aquaculture. The concentrations of elements with regulatory thresholds (Cu, Hg, Pb, Zn) were evaluated and this biochemical signature demonstrated potential of tracing production method of this species. Among these elements, only Pb presented concentrations consistently above the recommended limit for human consumption, particularly from will caught and earth pounds production. Using a chemometric approach based on multi-elemental signatures, sample origin was determined with an overall accuracy of 90%. Furthermore, a classification model was developed with samples from Olhão, and when applied to classify samples collected in Madeira Island it correctly allocated 27 of the 30 samples to its original production method (intensive sea cage aquaculture).

These results contributed to the development of traceability tools and their applicability in real case scenarios, as well as, regarding food safety, to raise attention to the potentially toxic concentrations of Pb for human consumption in fish from all origins and the high concentrations of As in wild-caught *S. aurata*. Thus, to our knowledge, the present study is the first that allowed to efficiently distinguish the production method of *S. aurata,* while additionally shedding light on its food safety features.

METABOLIC RESPONSES OF JUVENILE MEAGRE (*Argyrosomus regius*) REARED UNDER HYPO OSMOTIC CONDITIONS TOWARDS AQUACULTURE REFINEMENT

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The aquaculture of Meagre *Argyrosomus regius* an important commercial fish species, is still under development towards its full potential and optimized production Although the species main strengths are well known in fish farming namely high growth rate and feed conversion ratio, flesh quality, nutritional value, size, and husbandry techniques additional scientific research might generate rearing opportunities This species is migratory, spending their first months in estuaries, and then moving to coastal and offshore areas, constantly facing environmental differences that strongly determine their feeding activity, reproduction, and trophic migrations Nonetheless, meagre production has been restricted to sea cages and land salt based tanks Considering its high tolerance to a wide range of factors such as salinity, the present study aimed to assess the stress and biochemical costs associated with the rearing of juveniles under hypo osmotic environments, to better profit from this species characteristics and potentially settle them on diverse locations without productivity loss Organisms were reared for 28 days at salinity of 5 10 20 or 30 ppt, resembling estuarine and lagoon conditions. Growth rate was calculated, and biochemical responses associated with oxidative stress, oxidative damage, and energy metabolism were measured in muscle, liver and heart tissues, with limited effects detected in meagre for any of these conditions These results confirm the euryhaline characteristic of this species early life stages at the biochemical level, which are further on related to organism fitness, improving knowledge on future aquaculture techniques towards reduction of production costs and spread under diverse environmental conditions and practices, such as integrated multi trophic aquaculture or aquaponics.

SHORT-CHAIN FATTY ACIDS AS FUNCTIONAL INGREDIENTS TO BOOST EUROPEAN SEA BASS *(Dicentrarchus labrax)* GROWTH PERFORMANCE AND HEALTH STATUS

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Introduction

Novel aquafeeds usually include high levels of plant feedstuffs (PF). However, most PF has antinutritional factors (ANF) that may jeopardize nutrient digestion and metabolic utilization, increasing fish susceptibility to oxidative stress, intestine inflammation, and disease susceptibility. Recent evidence showed that short-chain fatty acids (SCFA) improve animals' digestive function, immunological and oxidative responses and modulate intestine microbiota. However, there is a lack of knowledge regarding single use or potential synergetic effects of SCFAs in fish fed with PF-rich diets. Therefore, the main goal of this work is to evaluate the potential of using SCFAs as functional ingredients in PF-rich diets on growth performance, immune status, and disease resistance of European sea bass, one of the most important fish species in the Mediterranean aquaculture.

Material and Methods

European sea bass juveniles (initial body weight 15.2g) were randomly distributed into twenty-one tanks (25 fish per tank) and fed in triplicate each experimental diet. Seven isoproteic (44% crude protein) and isolipidic (18% crude lipids) diets were formulated, including different short-chain fatty acids and different percentage supplementation: control diet with no supplementation and test diets with 0.25 or 0.5% of sodium acetate (SA), sodium propionate (SP), and sodium butyrate (SB). At the end of the growth trial, three fish per tank were sampled to evaluate their immune status.

After the growth trial, fish were relocated to the challenge room. Six fish per diet were randomly selected, and intraperitoneally (p.i) injected with 100ul of *Vibrio anguillarum* (1.2x10^{7} CFU/ mL^{-1}). Four and twenty-four hours after the p.i infection, fish were sampled to assess the hematological profile and innate immune parameters.

To assess the survival, fifteen fish per tank (thirty per diet) were also injected as previously described. These fish were fed the growth trial assigned diets, and the mortality was recorded for 3 weeks.

Data were analyzed for normality and homogeneity of variance and, when necessary, transformed before being treated statistically. Statistical analysis was performed with a three-way ANOVA, with time, fatty acid, and levels of supplementation as the main factors. When significant interaction occurred, one-way ANOVA for each factor was performed. The significant differences among means ($p<0.05$) were determined by Tukey's multiple range test. Non-orthogonal contrasts between control and other treatments were performed in each sampling time. All the statistical analysis was performed in the SPSS version 27.0 software package (IBM® SPSS® Statistics, New York, USA).

Results

At the end of the growth trial, dietary treatments had no effects on the growth performance and feed utilization efficiency of European sea bass juveniles.

During the challenge trial all blood parameters, decreased with time regardless of fatty acid or its level of supplementation comparing to time 0 (prior infection), except for mean corpuscular hemoglobin concentration (MCHC). All parameters showed a tendency to recover at 24h.

Mean corpuscular volume (MCV) is higher in fish fed sodium butyrate diets than in fish fed sodium acetate diets.

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In innate immune parameters, a time effect was found. Lysozyme and peroxidase activity decreased at 4 and 24h post-infection, compared to the other sampling times, regardless of the diet. This decrease may be related to the lower WBC values at 4h compared to time 0 and 24 h after p.i.

Peroxidase activity recovered at 24h in sodium acetate at 0.25 and 0.5% and at sodium propionate at 0.25% to similar values found in fish at time 0.

Lysozyme activity was higher in fish fed sodium butyrate when compared with sodium acetate, despite the inclusion level but was not different from the values found in fish fed sodium propionate diets.

Even though no significant differences in fish survival were observed between the tested and the control diets. Nevertheless, despite not being statistically different,fish fed the 0.5% sodium butyrate diets had the highest survival (93.3 % vs. 66.7% sodium propionate (SP) 0.25%).

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CHROMOSOME ANALYSIS OF EMBRYONIC AND LARVAL STAGES OF STERLET *Acipenser ruthenus*

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Introduction

The aim of the present study was to develop a routine cytogenetic method for the identification of ploidy level or chromosome aberrations in sterlet (*A. ruthenus*) embryos and larvae.

As chromosome engineering is the fundamental biotechnology tool that enables improvement and sustainable aquaculture of fish, including sturgeons, new analytical techniques are still required, especially for the early life stages of fish, including embryos (Chandra and Fopp-Bayat 2021; Fopp-Bayat and Kucinski 2022).

The presented procedure can be applied during chromosome manipulation in sturgeon fish with the efficiency of chromosome extraction ranging from 70 to 100% and the average number of recorded metaphases per slide ranging between 9 to 15. This protocol can be applied as a reliable tool in genome manipulations effectiveness or to check the correctness of the ploidy of experimental individuals.

Materials and methods

In the present study four age variants of sterlet were used: embryos 24 hours post-fertilization (HPF) (blastula stage), 4 days post-fertilization (DPF), 6 DPF (before hatching), and larvae with a yolk sac up to 7 days post-hatching (DPH). The experimental chromosome preparation procedure comprised several main steps, where all variables were optimized in a stepwise manner. The experimental variants of immersion of live embryos and larvae in 0.025% colchicine for 5 different time periods and different times of embryo hypotonization were experimentally tested. Chromosome slides were prepared in accordance with the standard splash procedure (Fopp-Bayat and Woznicki 2006).

Results and discussion

The developed rapid chromosome preparation protocol enabled the isolation of chromosomes from embryos and larvae of sterlet. The quality and quantity of chromosome slides were determined by tissue type, and the slides prepared from heads (gill cells) produced the most satisfactory results. Our results show that chromosomes can be isolated from every developmental stage of *A. ruthenus*, but the overall quality and quantity of the extracted chromosomes can vary. The most satisfactory metaphase plates were obtained from embryos at 6 DPH and larvae at the age of up to 7 DPH. In these groups, the quality of chromosomes supported counting and karyotyping. Chromosomes isolated from embryos of 24 HPF and 4 DPF were characterized by the weakest quantity and quality and could be applied for chromosome counting only. The optimal hypotonization period was 5-15 minutes for embryos at 24 DPF, 40 minutes for embryos at 4dpf, and 50-60 minutes for fish at 6 dpf-7 DPF.

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INNOVATIVE FISH FARM DESIGNS IN NORWEGIAN SALMON FARMING

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Introduction

The fast growth of aquaculture in recent decades has relied on several innovations (Asche, 2008; Kumar & Engle, 2016). Today, the need to achieve environmental sustainability is a strong driver for technological innovations (Osmundsen et al., 2020). To promote sustainable aquaculture and encourage relevant technological innovations, the Norwegian government launched free "development licenses" for salmon (*Salmo Salar*) production in 2015 (Norwegian Directorate of Fisheries, 2016). The industry responded by suggesting many different new farm concepts, providing a highly interesting insight into which directions the industry believes marine salmon production may take in the future (Føre et al., 2022).

Materials, methods and results

A systematic content analysis was performed to establish a base for investigating the technological directions proposed in the development license applications. The main material applied in the content analysis was the response and decision letters from the Norwegian Directorate of Fisheries (NDF) and the Ministry of Trade, Industry and Fisheries to the 104 applications for in total 884 development licenses.

By September 2021, 23 applications had been awarded, 78 had been rejected, and three applications had not received a final decision by the NDF. The awarded applications corresponded to 111 production licenses with a total maximum allowed biomass of 84,134 tons.

Categories of farm concepts were established based on observations in the applications for development licenses, including (listed by descending number of registered applications): Closed sea-based fish farms, conventional PE-rings, semi-submersible platforms, and rigid floaters. As many as nine closed farms concepts were awarded (39% of total awards), but only two PE-ring concepts (9%). Semi-submersible platforms and rigid floaters were the two concepts with the highest success rate in the allocation process: In both these categories, five of nine applications were awarded, representing 61% of the total allocated biomass.

Most suggested innovations were concerned with the prevention of sea lice infections, and all awarded concepts had measures against sea lice. According to the response letters, 70% of the applications and 87% of the awarded concepts involved specific measures against escape. 20 out of 23 were considered to contribute to the use of previously unsuitable locations (very sheltered and open ocean sites), or the increased sustainable utilisation of existing areas for aquaculture (coastal).

Some of these awarded concepts have been realized, followed by both success stories and challenges. Fish farmers report that they experience less parasitic sea lice on their fish, high water quality and optimal temperatures. This has for some resulted in good growth numbers, feed conversion factors, fish survival, and product quality. On the downside, fish escapes, demanding operational conditions (HSE-issues), and suboptimal water quality has also been reported. Time will tell which concepts will succeed in the long term, and if any will be able to compete with the currently dominating PE-rings. The development indicate that the future may include a variety of different concepts that are specially designed for environmental and biological conditions at the given sites.

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EFFECTS OF BIOACTIVE COMPOUNDS INCLUSION IN COMMERCIAL TYPE DIETS, ON GROWTH PERFORMANCE AND FEED UTILIZATION OF JUVENILES SEA BREAM (*Sparus aurata*) AND SEA BASS (*Dicentrarchus labrax*); PRELIMINARY RESULTS

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Introduction

Natural products rich in polyphenols have gained particular interest because of their plethora of biological activities and related health benefits for a wide range of farmed fish (Ahmadifar, et al., 2020). Over the last decade, protein hydrolysates from the microalgae *Spirulina (Arthrospira) platensis* containing bioactive peptides with biological activity are being studied as potential novel ingredients in functional foods for humans (Lafarga et al. 2021). The most important effects of polyphenols concern fish growth performance parameters and feed utilization, and overall fish health in intensive fish farming, while those of the bioactive peptides are still unknown in fish.

The aim of the present study was to evaluate the supplementation of different olive oil by- compounds rich in polyphenols and bioactive peptides from Spirulina, on growth performance, feed utilization, and fish health in European sea bass and Gilthead sea bream juveniles after they have been fed on a commercial like diet enriched with different % of these nutritional supplements versus a non-enriched diet. In the present abstract it is presented part of a more extensive study on the use of these bioactive compounds.

Materials and methods

European seabass (~9.1g) and seabream (~7.5g) juveniles, were fed three experimental diets each for 71 days at 24^{0}C water temperature, *at libitum*. All diets were isonitrogenous and isolipidic (54/16 protein/fat). Diets differed in their % of the supplements included, leading to 3 different diets per species: Diets 1, 2, 3 for sea bream, Diets 4, 5, 6 for sea bass and one control diet without any inclusion for each species of fish. The nutritional supplements added were olive leaf extracts or enzymatically treated with increased antioxidant hydroxytyrosol content, (Stymon Oliva & Stymon oliva treated), while the olive-pomace oil was enriched with hydroxytyrosol. Protein preparations of *Spirulina platensis* were enzymatically hydrolyzed to produce putatively bioactive peptides with molecular mass >30 kDa. Supplement % added in each diet are presented in Table 1.

Results

After 71 days of feeding, fish improved growth performance in diets 1 and 4 for sea bream and sea bass, respectively (Fig. 1). The same was observed in feed Efficiency although differences were not significant. The antioxidant status expressed as the activity of the enzyme Superoxide dismutase (SOD) of the fish, showed to be improved by the addition of the bioactive compounds combination in diet 1 for sea bream and 4 for sea bass.

Conclusions

In the current study, from the preliminary results it can be concluded that the addition of the combination of Stymon oliva, Stymon treated and olive oil pomace in combination with peptides derived from Spirulina, as in diets 1 for sea bream and 4 for sea bass, resulted in growth performance improvement of both species as well as in a better antioxidant status as the results of the antioxidant enzyme SOD showed.

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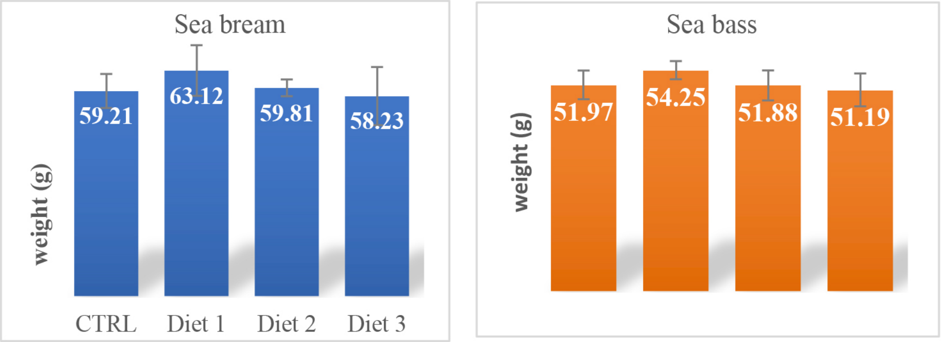

Fig 1 Weight increase for sea bream and sea bass juveniles at the end of the growth period

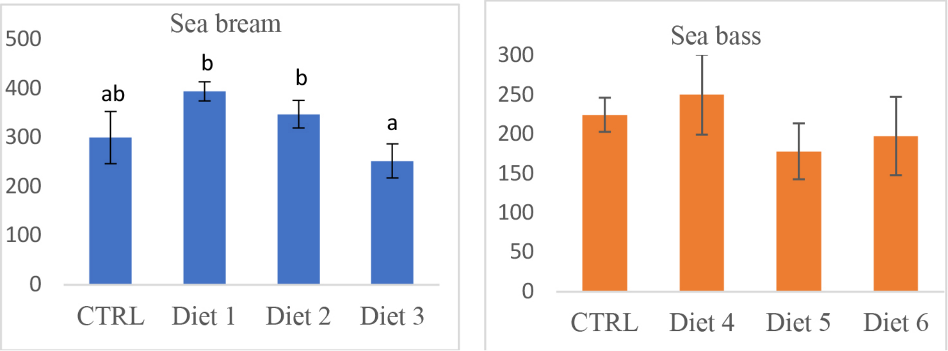

Fig. 2 Serum SOD U/ml in sea bream and sea bass juveniles at the end of the growth period

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GROWING UP STRONG – GENE EXPRESSION DURING THE EMBRYO-LARVAL-TRANSITION IN PIKEPERCH *Sander lucioperca*

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Introduction

Due to its delicate meat, high consumer acceptance, and a possible high reproduction rate, pikeperch is a new focus species in aquaculture (Policar *et al.*, 2019). However, several bottlenecks prevent the economically successful culture. In particular the early developmental stages between hatching and the larval development proved to be problematic and still difficult to rear, as they commonly exhibit high mortality levels. Early fish stages go through a sequence of developmental processes, which needs to be timed and balanced with endogenous resource use (Franz *et al.*, 2021; Franz *et al.*, 2022; Rombough, 2011; Wieser, 1995). The correct development of several tissues can hereby influence the survival success. In this context, muscle tissue development is crucial for early locomotion and ingestion abilities, as well as other physiological processes that may influence the specimens survival (China *et al.*, 2014; Muller *et al.*, 2000; Singleman *et al.*, 2012). Despite this, muscle development during this difficult early rearing, was not yet in focus for pikeperch.

Therefore, in this study, we analysed the expression levels of selected myogenic and developmental genes with the aim of providing a basis for future research on pikeperch muscle development.

Materials and methods

Commonly known gene markers for myogenic progenitor cells, myoblasts, and myofibres were selected (Watabe, 2001). In addition, developmental genes including skeletal markers were selected as they may influence muscle development (Sefton *et al.*, 2019). In this way, a total of 19 genes were examined at eight different developmental stages, ranging from early embryonic to larval stage (26 dd to 325 dd). Hereby, near-naturally reared specimens from a wild population were used. Each stage was analysed in triplicates with pools of 15 specimens using a nanofluidic qPCR approach. Relative gene expression was analysed using ANOVA procedure followed by a Tukey-Test to compare gene expression in between stages. The results were compared to the specimen development and previous literature on pikeperch ontogeny.

Results

The observed morphological development of the sampled pikeperches was consistent with previously present descriptions (Bastl, 1978; Güralp *et al.*, 2017). While the expression for myogenic marker genes generally varied, it tended to be elevated during three periods at the 53 dd (early embryonic), the 127 to 157 dd (before hatching), and the 280 dd stage (early larval stage) (Peňáz, 2001). The expression of developmental markers (e.g. MYH6 and EN2) was connected to ontogenetic processes. Furthermore, skeletal gene markers timed with skeletal development (Franz *et al.*, 2021; Franz *et al.*, 2022; Löffler *et al.*, 2008; Ott *et al.*, 2012) and corresponded with the observed second and third phase of elevated myogenic marker expression

Discussion

Analysis of the gene marker expression revealed the timing of three phases of increased myogenic gene expression. They occur around the somite formation (53 dd), before hatching (127-157 dd), and after the completed switch to exogenous feeding (280 dd). Specific levels of elevated marker expressions were found for each of these three phases, indicating initial basic muscle formation before the hatch and stronger growth and differentiation of muscle tissue during the last two analysed age groups. At the same time, two coinciding phases of skeletal marker expression were detected before the hatch and during the observed larval myogenic phase. The observations may support follow-up studies and help to improve muscle development and survival for pikeperches in aquaculture raise.

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BROWN SHRIMP *Crangon crangon* PROCESSING REMAINS ENHANCE GROWTH OF WHITE SHRIMP *Litopenaeus vannamei*

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Introduction

Brown shrimp, *Crangon crangon,* present the major coastal fishery in the southern North Sea with annual landings of up to 40,000 t (ICES, 2022). While only the abdominal muscle fraction is used for human consumption, the unused processing remains account for up to 60 % of the shrimp biomass. Incorporation of the remains into aquafeeds for high-valued species like *L. vannamei* would create by-product value, reduce waste, and promote circular economy approaches. This study aims to assess the suitability of brown shrimp processing remains (BSPR) as a sustainable feedstuff for *L. vannamei* to replace fishmeal. A detailed biochemical characterization was made and key apparent nutrient digestibility coefficients of BPSR examined. Controlled feeding trials with juvenile *L. vannamei* were conducted to determine effects on growth performance, feed utilization, digestive enzyme activities, and immunological parameters.

Materials and Methods

Frozen remains of mechanically peeled brown shrimp were obtained from a shrimp trader in northern Germany, dried at 60 °C, and ground to a homogenous meal. A detailed biochemical characterization of the meal was made using established methods (AOAC, 2010; Folch et al, 1957; Percot, Viton and Domrad, 2003) and by certified laboratories. To measure apparent nutrient digestibility coefficients, a reference and test diet was prepared using yttrium as inert marker. A 40-day feeding trial with 192 juvenile shrimp (6.5 ± 1.3 g) was conducted using pelleted diets replacing 0, 50 and 100 % of the fishmeal content with BSPR. Survival, molting frequency, growth, digestive enzyme activities, and muscle proximate composition of *L. vannamei* was determined. Based on the first trial, a second feeding trial with 720 shrimp (4.31 ± 1.0 g) was performed with six extruded feeds replacing the fishmeal content in steps of 20 % from 0 to 100 % to obtain an estimate for the optimal substitution level. The high water stability of the extruded pellets allowed for accurate measurements of feed intake and assimilation efficiency. Hemolymph was analyzed for phenoloxidase and prophenoloxidase activities and protein content.

Results and Discussion

Brown shrimp processing remains contain substantial amounts of key nutrients (521 $g\cdot kg^{-1}$ crude protein, 74 $g\cdot kg^{-1}$ total lipid, 15 $MJ\cdot kg^{-1}$ gross energy), minerals (phosphorus, magnesium, copper, manganese, selenium, zinc, and valuable functional ingredients (cholesterol, astaxanthin, chitin). Apparent digestibility coefficients of energy, protein, methionine, lysine, and copper exceeding 80 %, demonstrated excellent nutrient bioavailability of BSPR.

In the first feeding trial, shrimp showed best growth with diets containing a mixture of 50 % fishmeal and 50 % BSPR. No negative effects on muscle quality (moisture, protein, lipid) appeared and digestive enzyme activities were not affected by diet. The daily molting ratio increased with higher BSPR inclusion levels and a higher molting synchronicity was observed. The second feeding trial showed significantly enhanced growth when the BSPR substitution level exceeded 60 %. Based on the polynomial regression of the BSPR substitution level and shrimp weight gain, best growth is expected at 85 % inclusion level (Figure 1.). Different crustacean meals have been shown to stimulate growth in penaeid shrimp (Cordova-Murueta & Garcia-Carreno 2002; Williams et al. 2005; Nunes et al., 2019). This has been attributed to well-balanced nutrient profiles, increased palatability and even unknown growth promoters. Yet it remains unclear what the underlying mechanisms are. The shrimp hemolymph parameters (protein, phenoloxidase, prophenoloxidase) and digestive enzyme activities were not affected by the diets. Therefore, the enhanced growth performance might primarily be related to superior nutritional value and not immunological or digestive enzymatic improvements. We observed a reduced feed intake at concomitantly improved growth with diets containing 80 – 100 % BSPR. This supports the hypothesis that inherent component(s) of BSPR may promote growth in *L. vannamei*. Since the molting behavior of shrimp was also affected by the dietary treatment, it is possible that chitin-oligomers or chitin-protein derivatives in the BSPR are beneficial for shrimp growth. Good bioavailability and supply of key dietary nutrients further underline the suitability of BSPR as excellent feedstuff for *L. vannamei*.

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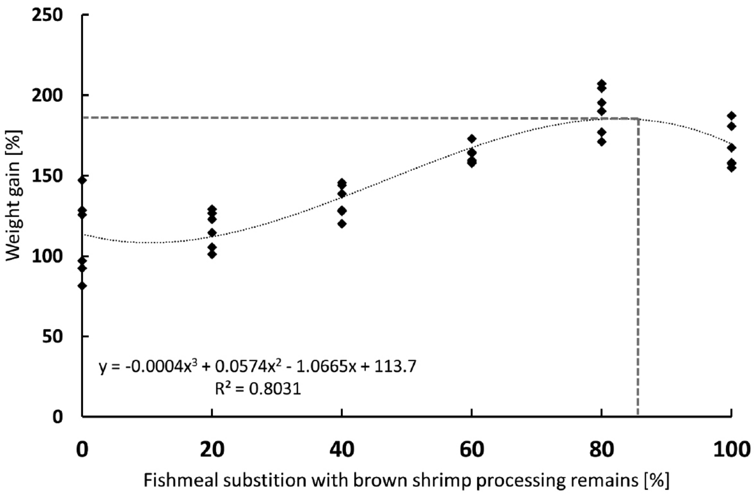

Figure 1. Polynomial regression between the amount of brown shrimp processing remains (%) and weight gain. Dashed lines indicate the local maximum weight gain (x= 85.2; y= 192.1)

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SOIL-BASED AQUAPONICS: THE EFFECTS OF SOIL ADDITION AS INVESTIGATED IN NINE DIFFERENT EXPERIMENTS

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Introduction

Under the new Commission Regulation (EU) 2018/848, which has entered into law in January 2022, aquaponic produce cannot be certified as organic in the European Union. Given the multiple components of an aquaponic system, which involve growing plants in hydroponic conditions, recycling of fish waste, and raising fish in artificial conditions, the achievement of organic certification for aquaponic produce is a complex matter dictated by many parameters. In the EU, aquaponics is considered a type of hydroponics, and mainly because of the lack of soil in its systems it is excluded from organic certification. This exclusion is considered to hinder the development of aquaponics, as it makes it difficult for producers to increase their earnings and effectively market their products. The research undertaken explores ways through which different soil substrates and potting mixes can be included in coupled and de-coupled aquaponics, and aims at helping aquaponics with its marketability, commercialisation, public acceptance, and popularity by bringing the technology one step closer to organic certification. The research was done through nine different experiments, which took place between April 2021 and August 2022. The experiments aimed at investigating the effects of soil inclusion on the health, growth, and quality of the plants and the fish, whilst examining how the soil microbiome is affected by the use of aquaponic water and aerobically-processed sludge. This presentation will include results, analyses, and conclusions for all the experiments. The experiments are listed below, with a short overview, results and conclusions for each.

Experiments 1&2: Coupled aquaponics of basil and Nile tilapia

Experiment 1 investigated the effects of aquaponic water on the growth and quality of basil (*Ocimum basilicum*), as well as potential effects of soil addition to fish health. The plants were grown in soil-filled pots placed in trays on top of fish tanks, creating full-recirculation, coupled aquaponic systems. The growth of the plants in soil-based aquaponic systems was compared to that of conventional, soil-less aquaponics ones. Two types of soil potting mixes were used, one with compost, and one without. The design of the systems was inspired by conventional coupled aquaponics, however the plant units were designed to allow the plant roots in soil-filled pots continuous access to fish water.

Experiment 2 was a continuation of the previous one, with a few modifications: compost amount was reduced, and the soil-less treatment was substituted by another soil-based treatment.

In experiment 1, the inclusion of soil was shown to be advantageous, as it prevented the appearance of nutrient deficiencies that instead showed in the soil-less treatment; in both experiments, compost produced higher yields than fish water, and the combined effect of compost and fish water produced the largest yield among all the treatments.

Experiment 3: De-coupled aquaponics of basil in pots

This experiment investigated the effects of aquaponic water and processed sludge on plant growth and quality, as well as soil microbiome composition in basil (*Ocimum basilicum*) grown in pots. The effects of the use of the different substrates and water types on plant health, quality, growth, as well as soil microbiome composition were investigated. The use of fish effluents as fertilisers was shown to be as effective as compost use, producing comparable yields.

Experiment 4: De-coupled aquaponics of onions in raised beds

This experiment investigated the effect of watering onions (*Allium cepa*) cultivated in raised beds and watered with water from different sources; the aim of the study was to compare the effects of manure, currently allowed in organic production, and fish effluents used as fertilisers on the growth and quality of the plants. Four outside raised beds were divided into four sub-units, and each subunit was devoted to the cultivation of onion sets. The four treatments were the following: onions watered with tap water, onions supplied with horse manure and watered with tap water, onions watered with aquaponic water, and onions watered with aquaponic water with sludge. Fish effluents were more effective than manure as fertilisers, producing larger yields.

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Experiment 5: De-coupled aquaponics of bok choy in certified topsoil

This experiment investigated the effect of watering bok choy (*Brassica rapa* subsp. *chinensis*) cultivated in detached containers in UK-certified topsoil and watered with water from different sources. The aim of the study was to compare the effects of manure, currently allowed in organic production, and fish effluents used as fertilisers on the growth and quality of the plants. The four treatments, each replicated three times, were the following: plants watered with tap water, plants supplied with horse manure and watered with tap water, plants watered with aquaponic water, and plants watered with aquaponic water and sludge. Fish effluents were more effective than manure as fertilisers, producing larger yields.

Experiment 6: De-coupled aquaponics of turnip in certified topsoil

This experiment investigated the effect of watering turnip (*Brassica rapa* subsp. *rapa*) cultivated in detached containers in UK-certified topsoil and watered with water from different sources. The methodology followed was identical to the methodology of experiment 5. At the time of writing, results had not been analysed yet.

Experiment 7: Use of barley for nutrient uptake of soil treated with fish effluents

This experiment investigated the use of barley (*Hordeum vulgare*) grown in the same raised beds where the onions from experiment 4 grew, in order to measure the uptake and retention of nutrients that would otherwise be washed off by rain by a cover crop. At the time of writing, the experiment was still ongoing.

Experiment 8: Coupled aquaponics of chilli peppers and Nile tilapia

This experiment investigated the effects of aquaponic water on the growth and quality of chilli peppers (*Capsicum annuum*) grown in soil-filled pots, placed in trays on top of fish tanks, creating full-recirculation, coupled aquaponic systems. Two types of soil potting mixes were used, one with compost, and one without. At the time of writing, the experiment was still ongoing.

Experiment 9: De-coupled aquaponics of chilli peppers in pots

This experiment investigated the effects of aquaponic water and processed sludge on plant growth in chilli peppers (*Capsicum annuum*) cultivated in pots. The effects of the use of the different substrates and water type on plant health and growth, and fruit quality were investigated. At the time of writing, the experiment was still ongoing.

SEASTAR MEAL AS AN ALTERNATIVE PROTEIN SOURCE IN DIETS FOR WHITELEG SHRIMP *Litopenaeus vannamei*

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Introduction

The global shrimp industry continues to grow revealing a farmed shrimp production of 6.0 Mt in 2018. *Litopenaeus vannamei* was the most produced species with 82.7% (FAO, 2020). Consequently, the requirement for formulated feeds globally increased to more than 8 million metric tons (Tacon, 2018; Boyd et al., 2022). The need to find more sustainable and low prized alternatives to fishmeal (FM) in shrimp diets remains a central challenge for the industry. Sea stars as a source of by-catch or by-product, harvested around mussel farms, offer an opportunity to improve the sustainability of formulated feeds. Sea star meal (SM) revealed a promising nutrient composition (van der Heide et al., 2018b) and was already used as a feed ingredient in pig and poultry diets (Afrose et al., 2016; Sørensen and Nørgaard, 2016; van der Heide et al., 2018a). However, there is no study about using SM in shrimp diets. Therefore, the aim of the present study was to determine the suitability of using meal of *Asterias rubens* to partially replace FM in diets for *L. vannamei*.

Materials and methods

Sea stars *A. rubens* were harvested in the Kieler fjord (Germany) and their nutritional profile was evaluated before formulating the diets. Shrimps were fed with four different diets including 0%, 5.4% (20% FM replacement), 13.5% (50% FM replacement) and 21.6% (80% FM replacement) SM for 42 days in a recirculating aquaculture system (Table I). All diets contained the same basal ingredients and were formulated according to an isonitrogenous and isocaloric content. Survival, growth performance and feed intake of *P. vannamei* were determined at the end of experiment. Additionally, haemolymph samples were taken to evaluate the health condition measuring metabolic parameters (glucose, total protein, triglycerides), number of haemolymph cells and the phenoloxidase activity.

Results

Sea stars showed an acceptable nutrient composition containing up to 44% crude protein and 9% crude fat. All experimental diets were accepted by the shrimps. Shrimps fed the 80% diet with highest SM content (21.6%) achieved even significantly higher growth performance (One-way anova, $p<0.05$) than shrimps fed the 0% diet or commercial control diet (Table II). Overall, growth of shrimps and feed intake were significantly higher in the formulated diet groups (0, 20, 50, 80%) compared to the performance of shrimps fed the commercial diet. Results of survival (Table II) and health performance, analyzed by the phenoloxydase acitivity, haemolymph cell counts, glucose, triglyceride and total protein levels in the haemolymph, showed no significant difference between shrimp groups fed the experimental diets.

Conclusion

Sea star biomass very efficiently replaces FM in diets for *L. vannamei* providing high-level protein and fat sources. In the current study, the inclusion of SM to a dietary level of 21.6% resulted in excellent growth and shrimp health. Future studies may test a 100% replacement of FM with SM. As the shrimp aquaculture industry continues to expand globally, sea star biomass joins a growing list of sustainable alternative protein and fat sources to replace FM. The authors encourage the application of this neglected marine biomass resource in aquafeeds.

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Table I Ingredients in % dry matter (DM) and nutrient composition of the four experimental diets fed to White leg shrimp *Litopenaeus vannamei*.

	0%	20%	50%	80%
Ingredients [%]				
Fish meal	27	21.6	13.5	5.4
Sea star meal	0.0	5.4	13.5	21.6
Soy bean meal	20.5	20.5	20.5	20.5
Shrimp meal	9.0	9.0	9.0	9.0
Wheat	30.1	28.2	25.6	22.9
Wheat gluten	1.0	2.7	5.1	7.5
Alginat	5.0	5.0	5.0	5.0
Fish oil	1.0	1.0	1.1	1.2
Soy lecithin	2.0	2.0	2.0	2.0
Vitamins and minerals	1.0	1.0	1.0	1.0
Lysine	0.7	0.8	0.9	1.0
Methionine	0.4	0.5	0.5	0.6
Calciumphosphat	2.0	2.0	2.0	2.0
Yttrium	0.1	0.1	0.1	0.1
Nutrient composition [%]				
Dry matter	88.8	89.4	88.6	90.8
Crude protein	41.6	41.6	41.4	41.3
Crude fat	8.4	8.5	8.3	8.5
Crude ash	11.7	12.5	13.8	15.0
Calcium	2.4	2.9	3.5	3.9
Phosphorus	1.7	1.6	1.4	1.2

Table II Growth performance and survival of Whiteleg shrimp fed four experimental diets and a commercial diet (control) for 42 days. Each value is mean ± S.D. (n = 4). Superscript letters indicate homogenous groups after pairwise comparison (Tukey HSD, α=0.05).

	Initial weight (g)	*Final weight (g)*	*SGR^{1} (% day^{-1})*	*Survival (%)*
0%	5.3 ± 0.35	**12.9 ± 0.80^{b}**	**2.11 ± 0.15^{b}**	95
20%	5.3 ± 0.50	13.3 ± 0.60^{ab}	2.18 ± 0.11^{ab}	100
50%	5.3 ± 0.41	13.6 ± 0.55^{ab}	2.23 ± 0.10^{ab}	100
80%	5.3 ± 0.37	**14.3 ± 0.42^{a}**	**2.36 ± 0.08^{a}**	100
Control diet	5.3 ± 0.55	**9.3 ± 0.78^{c}**	**1.32 ± 0.22^{c}**	100

[1] Specific growth rate (% body weight day^{-1})

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DUCK-AND-COVER AQUACULTURE, THE FUTURE OF MEDITERRANEAN AND MIDDLE EAST AQUACULTURE

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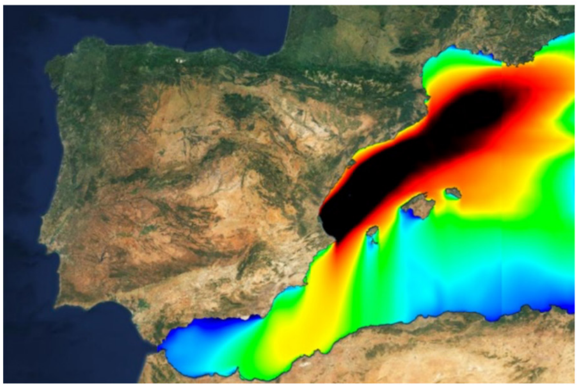
The growth of aquaculture in sheltered ocean waters has all but stalled due to issues such as environmental impact, stakeholder conflict, and available locations. As a result, open ocean aquaculture has grown significantly in the past 20 years. This expansion has fueled disruptive technologies that enable farmers to successfully produce fish in challenging conditions. A third and underdeveloped type of location stands to benefit from the breakthroughs in open ocean technology.

There are areas of opportunity throughout the globe where oceanic conditions require less robust equipment day to day yet still occasionally experience seasonal storms. These aperiodic events have proven to be catastrophic to producers every few years. A recent occurred on the Mediterranean Coast of Spain when storm Gloria devastated the industry in January 2020. Farmers reported a loss of more than 40% of sea bass and sea bream production and 60% of their Corvina yield.

Innovasea has been developing equipment suited for this market for the past five years. Based on more traditional equipment such as HDPE circular pens, the new line of equipment combines the functionality well known to farmers in these areas with the added ability to submerge during aperiodic storms such as Gloria. Essentially the equipment can "duck-and-cover" during the storms. The lower-cost offerings integrate with Innovasea's submerged feeding and remote mortality collection functions developed for the open ocean. Initial testing in Mexico shows promise for this technology in areas such as the Mediterranean and the Middle East.



EVALUATION *in vitro* OF PROTEIN BIOACCESSIBILITY OF MICROALGAE AND CYANOBACTERIA BY *Sparus aurata* DIGESTIVE ENZYMES

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Introduction

Microalgae, including some species of cyanobacteria, have the potential for reducing the dependence on conventional ingredients for aquafeeds, as they are a more reliable and less volatile source of protein. Overall, microalgae protein display well-balanced amino acid profile comparable to those of regular ingredients (Guedes *et al*., 2015). Despite that advantage, there are several drawbacks limiting the practical use of microalgae in aquafeeds. For instance, the presence of recalcitrant cell walls acting as protective barrier which hamper fish enzymes to access intracellular nutrients (Teuling *et al*., 2019). The efficiency of fish enzymes to hydrolyse microalgae cell walls relies on composition and branching of polysaccharide. In this regard, scarce research has been done to address how fish digestive enzymes hydrolyse microalgal protein (Tibbetts *et al*., 2017). The aim of this study was carry out a characterization of the protein nutritional profile and the *in vitro* protein bioaccessibility of marine (*Dunaliella salina*, *Nannochloropsis gaditana*) and freshwater (*Anabaena* sp., *Arthrospira platensis*, *Chlorella vulgaris*, *Spirogyra* sp.) microalgae and cyanobacteria, for assessing their potential as dietary ingredients for gilthead seabream (*Sparus aurata*) juveniles.

Material and Methods

Microalgae and cyanobacteria were evaluated in terms of total protein content and amino acid composition. In addition, protein bioaccessibility was estimated by means of a species-specific *in vitro* assay using *Sparus aurata* digestive enzyme extracts. To carry out the test, freeze-dried biomasses of six species of microalgae and two Cyanobacteria were used. Algal protein characterization was performed by elemental analysis (C:H:N). In addition, *in vitro* amino acid bioaccessibility was determined following the methodology described by Alarcón *et al*. (2001). At the end of the hydrolysis process, samples were withdrawn to carry out the quantification of total released amino acids and the determination of the specific amino acid profile by means of ion exchange chromatography with post-column derivatization with ninhydrin (Biochrom 30+ amino acid analyser, Biochrom LTD, UK) according to the manufacturer's protocol.

Results and Discussion

Overall, all the microalgae showed high protein content ranging from 25% to 61%. The highest values were found in *Anabaena* sp. and *D. salina* with 60.9% and 52.3%, respectively, whereas *Spirogyra* sp. showed the lowest (25.3%). All biomasses showed similar amino acid profiles, and although *Anabaena* sp. and *Spirogyra* sp. presented the highest and lowest absolute values, respectively, however, in relative terms, the proportions of amino acids were similar to that found in the rest of the species (Figure 1A). The *EAA/NEAA* ratio ranged from 0.91 to 0.95, which were higher than that of soybean meal (0.7), and within the range of fishmeal (0.92). The essential amino acids released after 90 min of *in vitro* proteolysis are shown in Figure 1B. The profile of amino acids released was clearly different among biomasses tested. Specifically, *Anabaena* sp. and *D. salina* showed the highest values of arginine released, whist the lowest values for this amino acid were obtained in *Spirogyra* sp. Moreover, *A. platensis* and *C. vulgaris* yielded relatively high levels of glutamic acid after the hydrolytic process, reaching, in the case of *A. platensis*, values up to four times higher than those observed for the rest of microalgae and cyanobacteria. Furthermore, *Anabaena* sp. had the highest tyrosine values, while *D. salina* showed high values of essential amino acids such as valine, leucine or lysine.

Conclusions

In the spite of the fact all the microalgae and cyanobacteria evaluated presented an adequate protein content and a balanced amino acid profile, the results obtained revealed differences in bioaccessibily of their protein to be hydrolysed by *S. aurata* digestive enzymes, and therefore in their bioavailability. Particularly, *A. platensis*, *N. gaditana* and *C. vulgaris* seem to be good candidates for feeding gilthead seabream.

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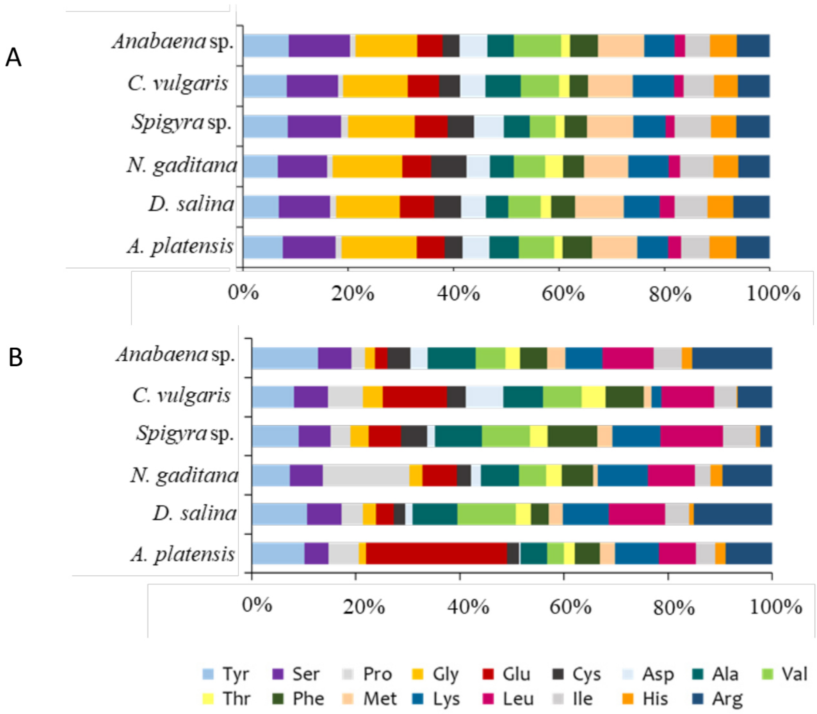

Figure 1. Amino acid profiles in the microalgae and Cyanobacteria biomasses evaluated (**A**), and profile of the amino acid released in reaction mixture at the end of the *in vitro* digestive simulation with *S. aurata* enzymes (**B**). Values are expressed in g amino acid 100 g analysed amino acids^{-1}.

Acknowledgements

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EFFECT OF DIETARY INCLUSION OF *Ulva rigida* OR A BLEND OF MICROALGAE ON DIGESTIVE FUNCTIONALITY IN TURBOT (*Scophthalmus maximus*)

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Introduction

Aquaculture faces changing and growing demands, among them the diversification with new species for satisfying market needs and improving economic performance of the sector. In this sense, turbot (*Scophthalmus maximus*) is an option for aquaculture farming systems owing to it is a carnivorous marine flatfish showing high growth performance and excellent fillet quality, which lead high commercial value (Wiriduge *et al*., 2020). However, turbot aquafeeds require a high protein content, which supposes up to 50% in the formulation. In this context, the research related to the use of new alternative ingredients for the aquafeed production has been increased. Algae have been positioned as interesting alternative to reduce the use of fishmeal in feeds for carnivorous fish (Geada *et al*., 2021). However, the effects derived from the dietary incorporation of different species of micro and macroalgae rely on the fish species considered, so each case should assessed specifically. Therefore, the main objective of this study was to evaluate the effect of the inclusion of *Ulva rigida* or a blend of microalgae on growth performance, feed utilization and digestive functionality in *Scophthalmus maximus* juveniles.

Material and Methods

The feeding trial was carried out at the Marine Culture Plant of the Spanish Institute of Oceanography (IEO, Santander, Spain). Experimental diets were elaborated at the Ceimar-Universidad de Almería facilities (Service of Experimental Diets, https://bit.ly/3wdO03h, Almería, Spain). Three experimental feeds **U5** and **M5**, containing 5% of *Ulva rigida* and 5% of a blend of microalgae, respectively, and a **CT** feed, which did not contain any algal biomass, were formulated. In addition, a commercial feed (**CTC**) was also tested. All diets were offered in triplicate to juvenile turbot during 60 days, and after that, nine specimens of each dietary treatment were sacrificed in order to extract the gastrointestinal tract. Intestine samples were obtained and processed for the study of digestive enzyme activities (trypsin, chymotrypsin and total alkaline protease, leucine aminopeptidase and alkaline phosphatase), and to analyse the ultrastructure of the intestinal mucosa by transmission electron microscopy (TEM).

Results and Discussion

The study of digestive enzymes can provide useful information about the nutritional status of fish (Alarcón *et al*., 1998). Figure 1 shows the results obtained after performing the analysis of the digestive enzyme activities. Overall, fish fed on U5 diet showed a general decrease in the enzyme activity levels compared to CT-fed fish, while fish fed on M5 showed the highest values. These results pointed out that the use of a blend of microalgae improves the intestinal digestion and absorption capacities in turbot juveniles. The morphology of the intestinal mucosa also plays a key role both in the absorption of nutrients and in providing a physical and immunological barrier against potential pathogenic microorganisms (Wilson & Castro, 2011). In this case, TEM images obtained showed a healthy mucosa in all the cases, without signs of damage or inflammation.

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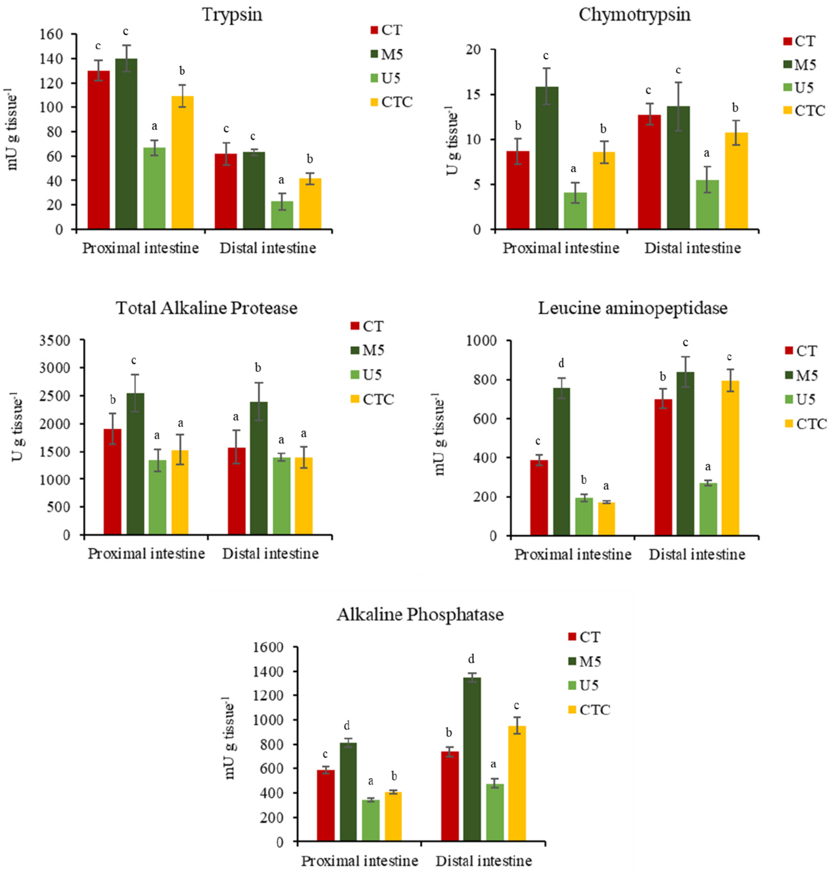

Figure 1. Digestive enzyme activities (U g tissue^{-1}) in proximal and distal intestine of *S. maximus* fed the experimental diets. Dietary codes: CT: control; U5: 5% *U. rigida*; M5: 5% microalgal blend; CTC: commercial turbot aquafeed. Values are expressed as mean ± sd (n=9).

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AUTOLOGOUS VACCINES AGAINST *Vibrio harveyi* AND *Tenacibaculum maritimum* FOR THE CONTROL OF INFECTIONS IN SEA BASS (*D. labrax*)

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Introduction

Among the bacterial pathogens affecting farmed European seabass (*D. labrax*), *Vibrio harveyi* and *Tenacibaculum maritimum* are recognised as noxious emerging agents, being responsible of frequent outbreaks and high mortality rates in the Mediterranean commercial farms. The infections are currently controlled by administration of antibiotic formulations and preventive measures including good aquaculture practices. Several studies have been dedicated to the *in vivo* evaluation of autologous vaccines efficacy against both pathogens, but none of them brought, up to now, to the development of patented commercial formulations (excluding a commercial vaccine against *T. maritimum* specifically addressed to turbot). The present study has been finalised to the tailoring of novel autologous vaccines against V. *harveyi* and *T. maritimum* to be tested within “in lab” and “in field” facilities, aimed at mitigating the disease impact on farmed European seabass.

Materials and Methods

Autologous vaccines against both pathogens were prepared at the Croatian Veterinary Institute of Zagreb starting from two bacterial strains recently isolated from spontaneous outbreaks of disease affecting local marine aquacultures. Preliminarily, the selected strains were submitted to phenotypical analysis and genome sequencing in order to identify/allocate them in the proper taxonomic position. Bacteria were cultured in Trypton Soy Broth and Marine Broth up to the log phase, respectively, then fixed with 0,4% formalin in order to get a whole-cell vaccine (bacterin). Formulations were subjected to sterility and safety test prior to the experimental use.

The **“lab” scale trials** were carried out using sea bass juveniles weighing approximately 10 grams divided in six experimental group (n=10) in triplicate, kept in fiberglass tanks, and subjected to the following treatments:

***V. harveyi* vaccination.** 1. Immersion for 1 min in 100ml vaccine (1.1x109 CFU/mL) + 900 mL sea water; 2. Intraperitoneal injection (IP) 0,1 ml/fish (1.1.x109 CFU/ml); 3. Untreated control groups (sea water as placebo for immersion and PBS for IP). ***T. maritimum* vaccination.** 1. Immersion for 2 min in 100 ml vaccine (3.75 x106 CFU/mL) + 900 mL sea water; 2. Intraperitoneal injection (IP) 0,2 ml/fish (6.0.x107 CFU/mL); 3. Untreated control groups (sea water as placebo for immersion and PBS for IP). After vaccination fish were submitted to organs and serum sampling for gene expression and immune response insights (ELISA for specific IgM), respectively. Moreover at 36 days post vaccination they were intraperitoneally or immersion challenged by the two bacterial pathogens in order to assess the relative percentages of survival (RPS) (vaccines efficacy testing).

The **“in field” scale trials** were carried out using sea caged sea bass juveniles (about 13 grams, n =1250/cage, 4 cages), and included the following treatments:
1. Vaccination by immersion for 1 min in the vaccine (3.3 x10^8 CFU ml^{-1}) against *V. harveyi* (dilution 1:10);
2. Vaccination by immersion for 2 min in the vaccine (7.4 x10^6 CFU ml^{-1}) against *T. maritimum* dilution 1:10);
3. Vaccination by immersion for 2 min in a mixure of both vaccines (dilution 1:10);
4. Untreated control group (PBS as placebo for immersion). After vaccination fish were submitted to serum sampling for immune response insights (ELISA for specific IgM). Moreover they were periodically surveyed in the field (sea cages) in order to assess their susceptibility to *V. harveyi* or *T. maritimum* natural exposure.

Results

Concerning the **“lab” scale** experiments, the potency testing (specific challenges) underlined the effectiveness of the proposed formulation. A better protection (highest RPS) was achieved after the intraperitoneal administration of *V. harveyi* bacterin, as well as after the immersion administration of *T. maritimum* bacterin. The ELISA based evaluation of immune response revealed a significant increase of specific IgM after the immersion vaccination with *V. harveyi* bacterin and a significant increase of specific IgM after the intraperitoneal vaccination with *T. maritimum* bacterin. The gene expression

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study allowed to observe significant upregulation of the genes coding for humoral immunity (IgT, p35) and also upregulation of some genes coding for cellular or non-specific immunity (IL1b, IL10). As regards the **"in field" scale** experiments, the ELISA based evaluation of immune response did not highlight specific findings to be correlated with the vaccinations performed. Still the fish submitted to the vaccinations with both autologous formulations or with the cocktail of them revealed an "in field" survival rate of 98%. Episodes of vibriosis and tenacibaculosis were reported only in the control unvaccinated sea cage, whereas in the vaccinated cages no outbreaks were recorded, and the periodical bacteriological samplings for the detection of *V. harveyi* or *T. maritimum* were always negative.

Discussion and Conclusions

The presents study, carried out within the AdriAquaNet project entitled "Enhancing Innovation and Sustainability in Adriatic Aquaculture" (INTERREG ref. number 1004516), allowed to set up a new and applicable protocol for the formulation and *in vivo* administration of immersion/intraperitoneal vaccines to *V. harveyi* or *T. maritimum*. The vaccination trials specifically targeted to sea bass juveniles showed that the novel autologous vaccines are safe and non-toxic and might be safely used in farm conditions. The vaccines also showed promising levels of protection against the two bacterial pathogens, both within experimental facilities and in farm conditions.

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POTENTIAL INCLUSION OF MACARONESIAN SEAWEED WRACKS IN DIETS FOR GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Stranding of macroalgal wracks that regularly appear on coasts from offshore seaweed beds play a key role in beach ecosystems. However, this clumping natural biomass is often interpreted as an indicator of beach poor quality by bathers, and it is usually removed and discharged under considerable economic costs. On the other hand, alternatives to fish oil and fish meal for aquafeeds must be found in order to improve aquaculture sustainability. The inclusion of algae in fish feed has been described to produce several physiological benefits such as an improvement in growth performance and lipid metabolism (Moutinho et al., 2018). Gilthead seabream (*Sparus aurata*) is a marine species with high commercial value and easy adaption to captivity whose production has increased in the last few years. Nowadays it is the most important finfish aquaculture product in the Mediterranean with a total production of 136,000 tons in 2020 (Savoca et al., 2021). The use of Macaronesian macroalgal wracks as a supplement in aquafeeds from a feasible ecological perspective is proposed in the present study.

Material and methods

For acclimatization, 228 juveniles of *S. aurata* (initial weight: 18.63 ± 1.00 g) were fed with the commercial control diet for 1 week. Then, fish were randomly divided in 12 tanks (1000 L) and fed three times per day with 3-5% of their total biomass, with one of four different diets in triplicate: (1) an extruded diet for gilthead seabream (Skretting) (CD, control diet); (2) CD supplemented with a 7% of a wind dried powder (1 mm) product of multispecific (MU) macroalgal wrack (30.9% *Lobophora* sp.; 21.9% *Dictyota* sp.; 19.6% *Asparagopsis* sp.; 17.5% *Cymopolia* sp.; 1.8% *Hypnea* sp.; 0.3% *Laurencia* sp.; 0.1% *Stypocaulon* sp., and 8.0% undetermined); (3) CD with a 7% of monospecific macroalgal wrack of *Lobophora* sp. (MOL) (>85%); (4) CD with a 7% of monospecific macroalgal wrack of *Dictyota* sp. (MOD) (>85%). After 93 days of feeding, 5 individuals of each treatment were slaughtered and specific growth rate (SGR), fish body indexes, hepatosomatic (HSI), viscerosomatic (VSI), and visceral-fat index (VFI) determined. Muscle samples were also collected for the analysis of proximate composition, lipid class (LC), fatty acid (FA) profiles and peroxides index (PI). TBARS and the activity of antioxidant enzymes were also measured in both muscle and liver. Finally, gut was removed in order to analyse the activity of digestive enzymes.

Results

Survival was 100% in all treatments. Fish growth was not compromised by the dietary inclusion of macroalgal wracks (SGR= ~1.40% day^{-1}). Proximate composition, HSI, VSI and VFI were similar regardless of the treatment. Muscle lipid composition was only affected in its higher content of monoacylglycerols in MU (1.43±0.30%) and MOD-fish (1.15±0.24%) *vs*. the other treatments (0.84-1.35%) and higher saturated FA (SFA) in MOD (26.36±0.55%) and MOL-fish (26.13±0.92%) compared to the other fish groups (24-25%). Antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) did not vary between treatments. Contrarily, glutathione reductase (GR) in muscle, presented the highest activity in MOD-fish (0.71 ± 0.09 *vs.* ~0.40 U mg protein^{-1}). Finally, digestive enzymes activities and oxidation status (PI and TBARS) of muscle remained unchanged regardless of dietary treatment.

Discussion and conclusions

Macroalgae are known to have anti-nutrient compounds that can reduce digestibility and nutrient absorption, possibly giving rise to reduced fish growth. Nonetheless, low seaweed inclusion (2.5-10%) can even improve growth and health performance in several species (Moutinho et al., 2018). It was expected that the lipolytic activity described for some brown macroalgae (Bourgougnon, 2014) caused a lower fat deposition in fish among other effects. However, growth, body indexes, proximate composition and digestive activities were unaffected by the different seaweed inclusion. This suggests that the basic composition of experimental diets may have met the nutrient requirements of gilthead seabream juveniles

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at the same level as the CD specifically designed for *S. aurata.* FA profiles did not vary, except for SFA, which increased in MOL and MOD-fish, as it has been previously described in *S. aurata* with a 5% of seaweed inclusion (Guerreiro et al., 2019). The lipolytic activity reported in macroalgae may be removing SFA from triacylglycerides in the liver for their subsequent storage in muscle. Seaweed supplementation may mitigate stress responses. However, it also increased lipid peroxidation in *S. aurata*, which the authors attributed to lipid layer degradation (Guerreiro et al., 2019). GR catalyzes the reduction of GSSG to GSH, representing the antioxidant restoration potential. Thus, the increment of GR activity in muscle of fish receiving the MOD supplementation suggests a higher capacity to mold the glutathione metabolism state by MOD treatment (Peixoto et al., 2016).

In summary, the inclusion of the MU, MOL and MOD in aquafeeds for *S. aurata* did not show negative effects on fish growth, perivisceral or liver fat deposition, proximate composition, main lipid profile or digestive capacity. A potential capacity to better restore the antioxidant status of the organism was detected with the MOD supplementation. As a result, the inclusion of a 7% of macroalgal wracks in diets for *S. aurata* may be feasible without apparent detriment in fish performance. Besides, it will contribute to the sustainable use of ocean resources and might empower the sustainable blue economy strategy in islands.

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GENOMIC ASSOCIATION STUDY FOR EARLY SEXUAL MATURATION IN A DOMESTIC STRAIN OF ATLANTIC SALMON

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Introduction

The early sexual maturation in the sea is a significant problem in cultures of *Salmo salar* and cause serious economic losses associated to low fillet quality among others. This phenomenon can be largely controlled, but not completely, by manipulating the photoperiod, being necessary to look other controls and recently it has also been possible through the identification and selection of genetic markers. Lochy is a domestic strain with rapid growth in cultures in the sea but that has reported with higher incidence of early maturation especially in males. According to recent studies the genomic signals between genotype and phenotype of maturation in Atlantic salmon (SNPs) are present even in 28 of 29 chromosomes and some maturation genes are *vgll3* in Ssa25 which has a great effect on the trait and other as *akap11, six6, maggi2 and picalm* (Ayllon et al. 2015; Sinclair-Waters et a. 2020). The objective was to identify QTLs associated with early sexual maturation in the Lochy strain of Atlantic salmon under the hypothesis that the trait is polygenic. For that we worked with data of 714 males obtained from 2 marine culture centers, genotyped with a microarray of 50k SNPs to later perform a genomic association analysis – GWAS, with the purpose to select them and reduce the negative effects of early maturation.

Materials and methods:

714 fishes, 80 mature and 634 immature males of the Lochy strain of *Salmo salar* were selected from two culture centers of “Salmones Camanchaca” company by a directed sampling focus on maturation and sexual secondary characteristics, in the processing plants located in the south of Chile. The culture was subjected to a continuous photoperiod of 24L: 0N from the transfer to the sea until the harvest, to prevent the early maturation. The samples were genotyped with a customized microarray of 50k SNPs and after we perform a genomic association analysis - GWAS using statgenGWAS of R. The ICSASG_v2 genome in the NCBI database was used to identify genes associated with maturation.

Results:

The sexual maturation observed in the targeted sampling varied between 2 (6/333) and 19% (74/381) in each center. The mature fish had a gonadosomatic index of 4.14 ± 1.67% and the immature ones 0.11 ± 0.33%. The GWAS revealed 34 significant SNPs for maturation in seawater (figure 1), which were distributed mainly in the chromosome 25 harbouring in the akap17A gene and nearly to *vgll3, chmp2b genes*. In contrast, only one or two QTLs were found linked to early maturation in Atlantic salmon on the other chromosomes. High variance explained for early sexual maturation was associated with two QTLs on chromosome 15 and 9. Candidate genes in these QTLs were associated a testicular development in male and cellular proliferation of embryonic cells in the presence of estrogen.

Conclusion:

With the QTLs detected in this study, the genetic architecture of sexual maturation in *Salmo salar* is deepened. These genomic regions of influence of sexual maturation in Lochy strain, allow us to select individuals that could be used to reduce the negative effects of early maturation.

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Table 1: 5 representative QTLs detected in the GWAS of sexual maturation in Lochy strain of *Salmo salar* and genes candidate identified in these regions.

SNP	Chromosome	p-value	Prop.Var	Harbouring gene	Nearest Genes
QTL1	15	6,06E-13	0,07	LOC106571007	*tcf21*
QTL2	9	6,18E-09	0,06	Intergenic region.	*fosb*
QTL3	1	5,81E-07	0,06	LOC106607942	LOC106607945
QTL4	26	1,52E-07	0,06	CD151 antigen-like	LOC106587085
QTL5	25	1,87E-08	0,04	akap17A	vgll3, chmp2b

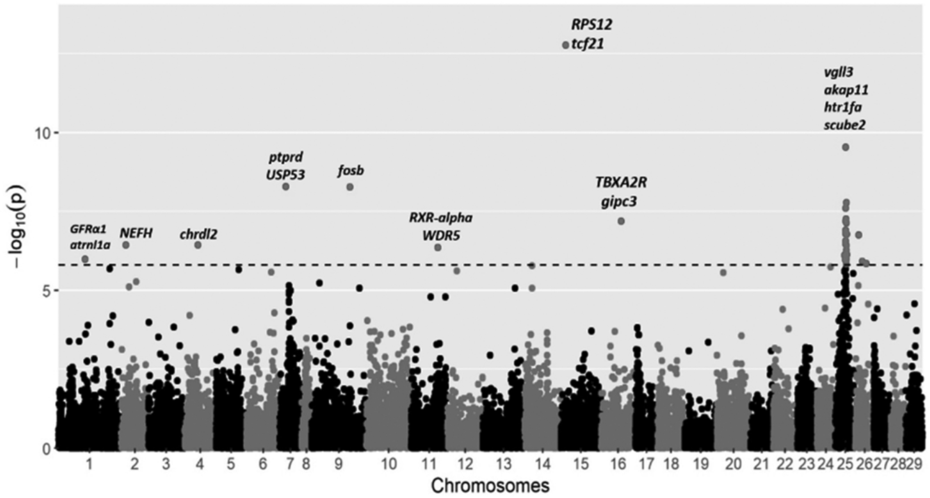

Figure 1: Manhattan plot with significant SNPs of sexual maturation of Lochy strain of *Salmo salar.*

Acknowledgment:

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INFLUENCES OF THE DESIGN AND MANUFACTURING OF VARIOUS BIOCARRIERS ON THEIR PERFORMANCE IN MOVING BED BIOFILM REACTORS (MBBR'S) IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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For optimal water quality management in RAS, a primary design concern is to provide the biofilter capacity required to control the total ammonia-nitrogen (TAN) concentration in the culture tanks. An optimal biocarrier will have a short start-up time- the time between when the biocarrier is added to the system and when the bacterial population can support the welfare of the needed biomass of aquatic species to be produced- and a high total ammonia nitrogen (TAN) removal rate during operation. The most challenging of these start-ups are in cold water systems, due to the slow development and metabolism of bacteria at lower temperatures. It is additionally challenging to 'cold start' a biofilter in a new system, where there is no existing 'mature' biomedia to be used for inoculation or seeding of the biological community. Experiments assessed the start-up time of five different designs of biocarriers in 14°C fresh water and, once nitrifying activity was established, their TAN removal capacity.

Five biocarriers of different designs, differing specific surface area (SSA) and protected surface area (PSA), sourced from 5 different suppliers were compared. SSA varied between 700 and 5500 and materials included virgin and recycled polyethylene, HDPE, and polypropylene. Experiments were carried out in 300L round tanks with an operational volume of 200L to ensure no exchange between tanks or loss of biocarriers through overflow and maintained at 14°C. Each biocarrier type was stocked at 50% of the operational volume (0.1m^3 per tank) in triplicate and hydrated for 48 hours before the trial start. Aeration was set at 20L/min/tank using blowers in the tanks. Following initial dosing to start biological activity in the tanks (NH_4CL (3.8g); $NaHCO_3$ (43g); $NaNO_2$ (0.4g); Na_2HPO_4 (0.08g) per tank) tanks were "fed" with calculated quantities of NH_4Cl and $NaHCO_3$ according to observed conversion rates, to promote ongoing nitrifying activity.

Over 10 weeks, DO; temperature; salinity; pH; KH; NH_4; NO_2; NO_3 data were collected at regular intervals using either probes (DO, temperature, salinity) or standard drop test kits, easily available to aquaculture producers, and with spectrophotometric titration.

There was found to be variation between hydration time required for the different biocarriers. Faster hydration times were related to faster start-up times. Start-up of bacterial development differed between biocarriers (2 weeks to 4 weeks) as did cumulative TAN conversion performance after start-up (week 4-10). Maximum daily conversion rates reflected the cumulative trend. Consumption of bicarbonate was found to be proportional to conversion of TAN and was higher than the typical values considered for a mature system, likely due to increasing conversion demand during the start-up phase. Start-up time and TAN conversion performance were not found to be correlated with SSA, contrary to common industry assumptions. Additionally, PSA was not related to rates of TAN conversion. Degassing affecting CO_2 levels; SSA vs. PSA; mixing rates; and the shape of holding tanks may have a greater influence than either SSA or PSA alone.

Selection of biocarrier for optimal cold start-up of RAS systems appears to be more complex than selecting that with the greatest SSA. Additionally, users should consider their production method and harvest strategy (e.g., continuous, batch all in/all out) when considering optimal biocarrier selection, as some may be more suitable where fast cold start-up times are needed, where others may take longer to start up but provide better and more stable continuous performance.

DEVELOPMENT OF A RAINBOW TROUT INTESTINAL PLATFORM FOR PROFILING THE ABSORPTION OF AMINO ACIDS AND SMALL PEPTIDES OF AN *IN VITRO* DIGESTED FEED

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Introduction

The generation of an artificial fish intestine could minimize the use of *in vivo* trails, which are presently necessary for testing each new feed, and are lengthy, expensive and require the use of many animals. Furthermore, although *in vivo* screening allows for a precise evaluation of the final effect of each feed, it does not improve our basic knowledge of the cellular and molecular mechanisms that determine the end-results obtained. This, in turn, severely limits our ability to understand and predict the biological value of a single ingredient and of their different combinations. To circumvent these constrains, we previously derived and characterized two novel stable cell lines from the proximal (RTpi-MI) and distal intestine (RTdi-MI) of the Rainbow trout (RT), demonstrating that the two cell lines retain the main properties of the tracts of origin. In the present work, we used these cells to set up a barrier developed on a commercial permeable (PET) membrane support, to profile the absorption of dietary amino acids of an in vitro digested fish feed.

Materials and methods

In vitro digestion

A commercially relevant RT feed was produced by Skretting Aquaculture Innovation with crude protein and lipid level of 46 and 23%, respectively. In order to expose the feed to the gastric and intestinal digestive conditions, in vitro digestion (IVD) was performed using either RT stomach or intestinal enzyme extracts at either acidic or alkaline conditions. After preparation, the *in vitro* digested samples (IVD) were lyophilized for 24 hours and stored at -20°C until performing cell assays.

In vitro cell assays

The intestinal epithelial cell lines RTpi-MI and RTdi-MI were seeded at 250.000 cells/cm^2 onto cell culture inserts (Greiner BioOne, ThinCert cat. No. 665640) and cultured at 20°C under ambient atmosphere. Trans epithelial cell resistance (TEER) was measured using an EVOM2 Epithelial Voltohmmeter with STX2 electrode (World Precision Instruments). TEER was calculated by subtracting the values without cells from the values with cells. Four combinations were tested: 50% and 25% (vol/vol) of IVD diluted in Hanks′ Balanced Salt solution (HBSS), with or without 4 mg/mL Bovine Serum Albumin (BSA), all spiked with 100 μg/mL 4 KDa FITC-Dextran. 500 ul of each combination was pipetted in the apical compartment of the inserts, and 1.2 mL HBSS was pipetted into the basolateral compartment. After 6- and 24- hours of incubation, 0.1 mL samples were collected from the apical and basolateral compartments of the PET membrane support. FITC-Dextran was quantified using a Synergy HT Multimode microplate reader (BioTek EX) to assess the integrity of the epithelial barrier during the experiment. Thereafter, epithelial barriers were rapidly fixed using 4% paraformaldehyde and stained with hematoxylin and eosin to evaluate cell morphology and for endogenous Alkaline Phosphatase (AP) activity to detect fully differentiated enterocytes.

Biochemical analysis

Amino acid and small peptide profiles were evaluated by targeted and untargeted approaches in a single run by hydrophilic interaction liquid chromatography tandem Exploris-Orbitrap®- high resolution mass spectrometry (HILIC-Exploris-HRMS). The following 14 amino acids were quantified: L-Alanine, L-Arginine, L-Glutamine, L-Isoleucine, L-Leucine, L-Lysine, L-Asparagine, L-Methionine, L-Phenylalanine, L-Threonine, L-Tryptophan, L-Tyrosine and L-Valine. The

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amount of each amino acid in the apical and in the basolateral compartments was compared to the control IVD (sample not incubated with cells). Absorption of each amino acids was expressed as the percentage of the amino acid found in the basolateral compartment/amount quantified in the control IVD.

Results and discussion

At day 7 of culture, both RTpi-MI and RTdi-MI cells formed an effective epithelial barrier, as indicated by reaching plateau values of TEER (RTdi-MI: 30.5 ± 1.6 Ohm*cm^2; RTpi-MI: 28.9 ± 1.9 Ohm*cm^2).

After exposure to 25% and 50% IVDs with and without BSA, the integrity of the epithelial barrier was not affected in both cell lines, as indicated by the constant TEER values. In agreement with these results, after all treatments, both RTpi-MI and RTdi-MI cell barriers retained Dextran 4KDa in the apical compartment of the inserts at a significantly higher rate than the inserts without cells. Morphological analysis confirmed the presence of an intact cell monolayer on the membrane, further confirming that IVD incubation did not alter the epithelial barrier functionality after 6- or 24- hour incubations.

Biochemical analysis showed that all 14 amino acids were detected in all 4 combinations tested (25% and 50% IVD HBSS, with and without BSA) in the apical and in the basolateral side of both RTdi-MI and RTpi-MI cell barriers after 6- and 24- hour incubation, confirming the ability of cell lines to absorb different categories of amino acids. Absorption of all the amino acids after 24-hour incubation was higher compared to 6-hour incubations with all the media combinations tested. After 24-hour exposure to 50% IVD without BSA, the amount of arginine, glutamine, histidine, valine, methionine and threonine in the apical side of the RTdi-MI cell barrier significantly increased compared to the apical amount quantified in any other experimental group, while no differences among groups were recorded for the remaining amino acids. Consistent with these results, small peptides enriched with arginine, glutamine, histidine, valine, methionine and threonine were present in the apical compartments of both cell barriers after 6-hour incubation of all the media combinations. Whereas these peptides were not present after 24-hour exposure of RTdi-MI cell to 50% IVD HBSS without BSA, suggesting that the amino acid increase was likely due to the digestion of the small peptides in this treatment. Furthermore, these amino acids reached the equilibrium between the apical and basolateral compartments after 24 hours only when 50% IVD was supplemented with BSA implying that this protein may have a functional role in their absorption.

We also observed that RTdi-MI cells were more differentiated towards mature enterocyte than RTpi-MI cells, as shown by the detection of a higher alkaline phosphatase activity.

Conclusions

Overall, our results indicate that both RTpi-MI and RTdi-MI cells can absorb the amino acid components of pelleted feed digested *in vitro*, though with some difference between them. RTdi-MI cells, 50% IVD HBSS and 24 hour- incubation seems to be the most efficient combination, although the role of BSA needs to be further investigated. Moreover, this cell line showed the ability to cleave some small peptides increasing arginine, glutamine, histidine, valine, methionine and threonine availability for further absorption. In conclusion, the simple platform used here represents the initial step towards the generation of a more complex alternative that may improve the differentiation of the RT cell lines, thereby leading to a better reproduction of the physiological environment distinctive of the *in vivo* native tissue.

Supported by European Union’s Horizon 2020 research and innovation programme under grant agreement No 828835

EFFECT OF MACROALGAL POLYSACCHARIDES ON IMMUNE RESPONSE & SUSCEPTIBILITY TO EDWARDSIELLA TARDA IN OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Introduction

Polysaccharides are well-known for their range of biological activities, such as antibacterial, antiviral or prebiotic properties and immune-stimulant effect on animals and humans (Hindu *et al*., 2019; Ngo and Kim, 2013). Sulfated polysaccharides from green macroalgae modulate the transcription of immune mediators involved in defense mechanisms within the innate and the adaptive immune response (Berri *et al.,* 2016). Hence, they could be of interest to enhance the resistance of fish to pathogenic diseases that are frequent in modern production, such as *Edwardsiella tarda*.

Materials and methods

Diets and feeding trial

Four hundred and eighty juvenile Olive flounder (26.51g ±0.02) were acclimatized in 300L polyvinyl circular tanks supplied with filtered seawater at a flow rate of 10L/min and aerated with air-stones to maintain sufficient dissolved oxygen at the Jeju university experimental facility. After two weeks of acclimation with a commercial diet, fish were fed with 3 different experimental diets to apparent satiation twice a day (08:30h and 18:00h) for 12 weeks: a basal diet (CON); and a basal diet with two inclusion rates of mix of macroalgal polysaccharides: 3g/kg (AL3) and 5g/kg (AL5). All experimental diets were extruded and using sardine fish meal (55%), soybean meal (12%), wheat flour (19.7%), wheat gluten, soy protein concentrate, tangkage meal, poultry by-product, fish oil, vitamin and mineral premixes. The bacterial challenge was conducted between day 86 and day 101 in 120 L acryl tanks. Fish were intraperitoneally injected with a *E. tarda* strain at LD50 (1×10^5 CFU/ml) and mortality was monitored and recorded every 2 hours for 21 days.

Sample collection and analysis

Fish performance: feed conversion ratio, average body weight and survival rate were recorded after 6, 9 and 12 weeks before challenge. Immunological parameters were analyzed before challenge by collecting blood samples from three fish per tank: total immunoglobulin (Ig) according to the method described by Siwicki and Anderson (1993), lysozyme activity by turbidometric method described by Hultmark (1980), antioxidant parameters (superoxide dismutase (SOD) and catalase using kits from Sigma-Aldrich 19160 and Biovision, Inc. California, USA). Intestinal health was evaluated through counting of mucus-secreting goblet cells (GC) in the whole intestine from two fish per tank after 24h of starvation. Results were analyzed by ANOVA and the differences in mean values were compared using Tukey's HSD test ($P<0.05$).

Results

In the current study, the growth performance of fish was not significantly affected by the additive.

Dietary supplementation of algae extract improved the survival of fish challenged with *E. tarda*, which likely resulted from the enhanced innate immunity and activities of antioxidant enzymes. Survival percentages in CON, AL3 and AL5 groups were 48.9 ± 3.85, 73.3 ± 0.00 and 80.0 ± 6.67 respectively (*Figure 1*).

Lysozyme is one of the well-known enzymes of the innate immune response representing the bacterial killing-activity of immune cells through hydrolysis of peptidic bonds in the bacterial cells. SOD and catalase are well-known antioxidant molecules produced to prevent the deleterious effect of stressors (pathogenic or environmental). The use of the mix of macroalgal polysaccharides significantly increased these values compared to control at both concentrations *(Figure 2).*

Additionally, both dietary groups supplemented with algal polysaccharides improved intestinal morphology and led to an increase in goblet cell counts (*Figure 3*).

Conclusion:

The findings in this study indicate that in-feed macroalgal polysaccharide supplementation modulates immunity and strengthens gut barrier function which resulted in a better fish resistance and finally strongly improved the survival of fish exposed to *E. tarda* (up to 63% survival rate 21 days post challenge)

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FIGURE 1. The survival of olive flounder challenged with *Edwardsiella tarda* (1 × 10^5 CFU/ml).

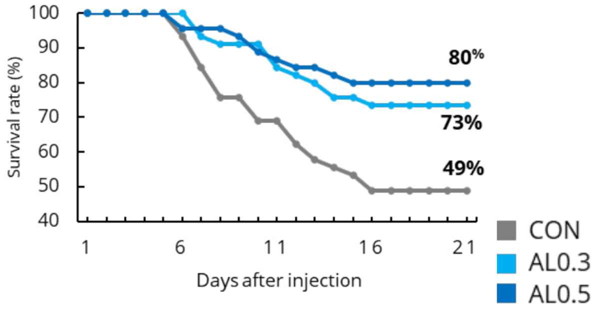
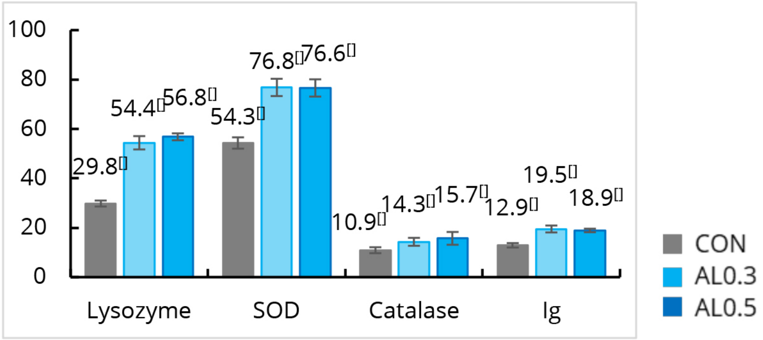
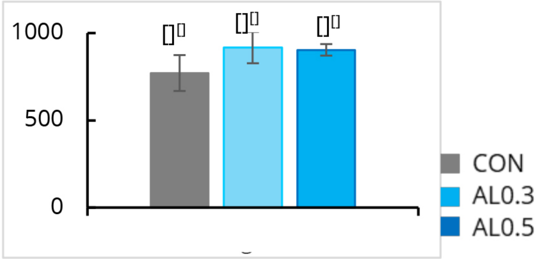


FIGURE 2. The innate immune response and antioxidative enzyme activities of olive flounder after the 12-week feeding trial.



Values are mean of triplicate groups and presented as mean ± SD. Different superscript letters are significantly different ($p < 0.05$). Abbreviations: AL3, algae extract 0.3%; AL5, algae extract 0.5%; CAT, (catalase activity [mU ml−1]); CON, control; Ig, (total immunoglobulin [mg ml−1]); LYZ, (lysozyme activity [µg ml−1]); SOD, (superoxide dismutase [% inhibition]).

FIGURE 3. Goblet cell counts in the intestine of olive flounder fed the four experimental diets for 12 weeks.



Values are mean of triplicate groups and presented as mean ± SD. Values with different superscripts are significantly different ($p < 0.05$). Abbreviations: AL3, algae extract 0.3%; AL5, algae extract 0.5%; CON, control.

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Berri, M., Slugocki, C., Olivier, M., Helloin, E., Jacques, I., Salmon, H., Demais, H., Le Goff, M., & Collen, P. N. (2016). Marine-sulfated polysaccharides extract of Ulva armoricana green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. Journal of Applied Phycology, 28(5), 2999–3008. https://doi.org/10.1007/s10811-016-0822-7

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EFFECTS OF DIETARY MARINE SULPHATED POLYSACCHARIDES (ALGIMUN®) ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND DISEASE RESISTANCE OF JUVENILE GILTHEAD (*Sparus aurata*) TO *Photobacterium damselae sbsp piscicida*

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Introduction

Dietary seaweeds and their bioactive components have positively affected some fish species' health parameters and growth performance (Thépot *et al.,* 2021). Recent work has pointed out the potential of in-feed marine macroalgal polysaccharides as reliable agents for the modulation of the immune function and the reinforcement of the intestinal barrier function of animals. Sulfated polysaccharides from green macroalgae modulate the transcription of immune mediators involved in defense mechanisms within the innate and the adaptive immune response (Berri *et al.,* 2016). Hence, they could be of interest to enhance the resistance of fish to pathogenic diseases that are frequent in modern production, such as *Photobacterium damselae* sbsp. *piscicida*.

Materials and methods

Diets and feeding trial

A total of two hundred and forty juvenile gilthead seabream (6.00 ± 0.03g) were randomly separated into 12 tanks (400 L, 20 fish per tank) distributed in four replicates and reared in a recirculating system. For the 30-day feeding trial and the 14-day post-bacterial challenge period, feeding was done three times a day, seven days a week at 3% body weight (Morning: 1.4%, Midday: 0.6%, Evening: 1%). The three quadruplicate treatments received an experimental diet with different inclusion levels of Algimun®: Control (C), AL0.3, and AL0.5 with Algimun® at 0, 0.3 and 0.5 g kg^{-1} diet, respectively. All experimental diets were extruded. The diets were formulated with dietary fish meal, plant ingredients, fish oil and vitamin-mineral mix. After 30 days of the feeding trial, all fish from each group were injected intraperitoneally with 100 µL PBS containing 1×10^7 CFU *Photobacterium damselae* sbsp. *piscida* strain cells. *P. damselae* sbsp. *piscida* was re-isolated to confirm the mortality due to the bacterial infection. Mortality was recorded for eight days until mortalities had ceased, and the observation of surviving fish was extended to 2 weeks.

Sample collection and analysis

Fish performance: Feed conversion ratio, specific growth rate and survival rate were calculated. Immune response: At 10, 20 and 30 days of the feeding trial (pre-challenge), blood samples were collected for total myeloperoxidase (MPO) quantification and lysozyme activity (LYS). The total Myeloperoxidase (MPO) content was measured on plasma according to the method described by Quade and Roth (1997) and lysozyme activity was determined according to Ellis (1990).

Results

The final mean weight (FMW), specific growth rate (SGR), and feed conversion ratio (FCR) of fish fed with AL0.3 and AL0.5 diets were significantly improved compared to fish fed with the control diet ($P<0,05$). However, there was no statistical difference between the marine sulphated polysaccharide groups (*Table 1*).

TABLE 1. Growth performance and economic parameters of gilthead seabream fed with different doses of Algimun®.

Survival against *Photobacterium damselae* subsp. *piscicida* was numerically increased in both seaweed-based dietary groups compared to the control group (>20%) (*Figure 1*). The higher survival rate in the treatment groups, especially the AL0.5 group, is in line with the improvement of the innate immune responses (*Figure 2 and Figure 3*).

FIGURE 1. Long-range and survival rate (%) of gilthead seabream fed control (C) and diets containing Algimun® (AL0.3 and AL0.5).

Myeloperoxidase is produced by neutrophils and triggers a release H_2O_2, which is highly toxic for pathogens. The myeloperoxidase activity (MPO) was significantly higher in AL0.5 compared to other dietary groups on each sampling time ($P < 0.05$) *(Figure 2)*.

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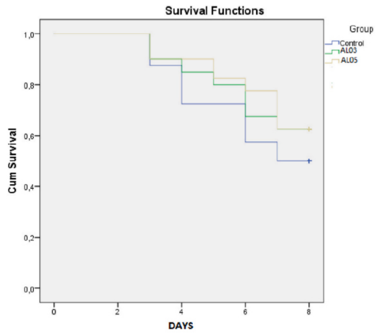
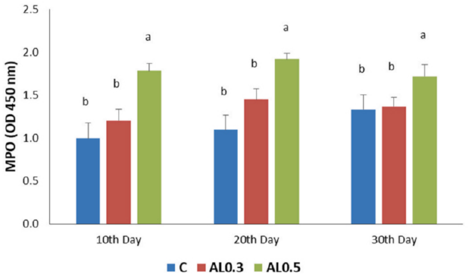
	C	AL0.3	AL0.5
Initial mean weight (g)	6.00 ± 0.03	6.01 ± 0.05	6.03 ± 0.06
Final mean weight (g)	10.86 ± 0.05^b	11.38 ± 0.01^a	11.50 ± 0.04^a
Feed conversion ratio	1.65 ± 0.01^a	1.49 ± 0.04^b	1.47 ± 0.06^b
Specific growth rate (%/day)	1.98 ± 0.03^b	2.13 ± 0.03^a	2.15 ± 0.04^a
Economic conversion ratio ($/kg)	1.82 ± 0.01^a	1.64 ± 0.04^b	1.63 ± 0.06^b
Economic profit index ($/fish)	43.25 ± 0.15^b	45.79 ± 0.14^a	46.31 ± 0.37^a

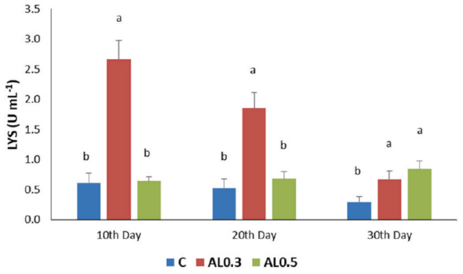
Different superscript letters indicate significant differences between groups ($p < 0.05$).



Overall comparisons of test of equality of survival distributions for the different levels of the group were performed with Log Rank (Mantel-Cox) test (Chi-Square: 2.077, df: 2, and Sig. 0.354).



Different superscript letters indicate significant differences between groups ($p < 0.05$).



Different superscript letters indicate significant differences between groups ($p < 0.05$).

FIGURE 2. Myeloperoxidase activity (MPO) of gilthead seabream fed with experimental diets containing different levels of Algimun® as 0 (C), 0.3 (AL0.3) and 0.5 (AL0.5) g/kg.

Lysozyme is an essential enzyme that lysis the pathogenic bacterial cell wall. In the present study, lysozyme activity (LYS) was significantly higher, when compared to the control group in the AL0.3 group at all sampling times and in the AL0.5 group at day 30 ($P < 0.05$).

FIGURE 3. Lysozyme activity (LYS) of gilthead seabream fed with experimental diets containing different levels of Algimun® as 0 (C), 0.3 (AL0.3) and 0.5 (AL0.5) g/kg.

Conclusion:

Dietary marine macroalgal polysaccharides increased growth performance, decreased FCR and improved the resistance of fish leading to an increase in the survival rate against *Photobacterium damselae* sbsp. *piscida*. This in-feed strategy acted as an immunomodulator and proved its efficiency in protecting and defending aquatic animals' health.

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EFFECTS OF FUNCTIONAL DIETS BASED ON MICRO- AND MACROALGAE ON THE INTESTINE RECOVERY OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

It is recognized that nutrition is a key modulator of fish digestion and gut integrity. The structure and function of the intestine play a vital role in fish digestive capacity and efficiency. However, the digestive system is affected by different biotic and abiotic external factors (e.g antinutritional factors of the feed ingredients, stress situations during farming). Innovative and sustainable production strategies are required to assist the growth of the aquaculture industry. Functional diets can be defined as those that when supplemented confer specific benefits such as physiological functions or enhance fish health. Studies of low inclusion levels of micro-and macroalgae promoted fish growth, feed utilization, and physiological functions (gut microbiota, antioxidant activity, and stress response). In this study, we evaluated the effects of the inclusion of *Phaeodactylum tricornutum* and *Gracilaria gracilis* in gilthead seabream (*Sparus aurata*) diets to study fish intestine recovery after provoked intestinal inflammation. Physiological responses to the inclusion of micro-and macroalgae species were assessed through the integration of data from nutrient absorption and utilization and enzyme activity.

Material and methods

- *Diets*

A control diet (CTRL) was formulated to be like a commercial feed for gilthead seabream juveniles. The remaining experimental diets were formulated by supplementing the CTRL diet with microalgae (*Phaeodactylum tricornutum*; PHA) or macroalgae (*Gracilaria gracilis*; GRA) at 2.5%, or a blend of micro- and macroalgae at 5% (50:50; BLEND). Experimental diets were manufactured at SPAROS Lda. (Olhão, Portugal). *Phaeodactylum tricornutum* was obtained from Necton S.A (Olhao, Portugal) and *Gracilaria gracilis* from Alga+ (Ílhavo, Portugal).

- *Fish rearing*

Experiments were carried out in compliance with the Guidelines of the European Union Council (Directive 2010/63/EU) and Portuguese legislation for the use of laboratory animals. Gilthead seabream juveniles were obtained from Atlantik Fish Lda. Portugal and the experiments were conducted at the Ramalhete Experimental Research Station of the Centre of Marine Sciences (CCMAR, Faro, Portugal).

- *Assisted-feeding with saponins*

To study the intestine recovery after an insult in gilthead seabream juveniles, and the possible recovery through nutrition, the treatments were: a positive control (PCTRL) where fish were assisted-fed with two empty jelly capsules, afterwards fed with the CTRL diet. For the remaining treatments, fish were assisted-fed with two jelly capsules filled with soy saponins (n = 2; 850 mg saponins). Fish from negative control (NCTRL) were fed the CTRL diet during the trial. Fish from PHA treatment were fed the diet containing *P. tricornutum* at 2.5%. Fish from GRA, were fed the diet containing *G. gracilis* at 2.5%. In the fifth treatment BLEND, fish were fed the diet with micro- and macroalgae at 5%. Gilthead seabream juveniles with a mean body weight of ±176.06 g were distributed into 15 cylinder-conical 500 L tanks at an initial density of 9.8 kg/m^3 (28 fish per tank). After 24 h of fasting, fish were anaesthetized and transferred, using a fish net, onto a dry plastic tray. Two jelly capsules were inserted into the fish's stomach and gently pushed directly into the oesophagus using a solid piece of plastic. After the assisted-feeding each fish was placed into airflow and clean water and monitored for eventual capsule regurgitation. Once recovered, the fish was transferred into their respective tanks. After a period of 72 h without feeding the fish, each experimental diet was randomly assigned to triplicate tanks and fed for 20 days. Fish were fed by hand to apparent satiety twice a day from Monday to Friday and once a day on Saturday.

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- *Sample collection & analysis*

At the end of the trial, after 24 h of fasting each tank was bulk weighed and counted. Five fish from each tank were euthanized and individually weighed. Blood was withdrawn individually from the caudal vein of five fish. For histology analysis, approximately a 1 cm sample of the anterior intestine was dissected. Samples for intestine histology are under analysis.

- *Blood plasma enzymes analysis*

The activities of enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined in plasma. Samples were run at 30ºC in duplicates using a multimode microplate reader (BioTek Instruments, Inv. USA) and commercial kits (SPINREACT, S.A, Spain).

Results and Discussion

Blood plasma enzymes analysis

Alanine and aspartate aminotransferases were not affected by the dietary treatments ($p>0.05$). The ratio ALT:AST that could indicate a possible liver damage or the ratio AST:ALT that could identify a possible disorder in other organs were also not affected by the treatments. The fish fed the BLEND diet presented a significant ($p<0.05$) lower ALP when compared to fish from remain dietary treatments. A higher ALP could in some cases indicate an anti-inflammatory response situation. This result could indicate a possible positive regulation of the BLEND diet in gilthead seabream gut health. The results from anterior intestine histology could shed some light on intestine recovery responses in gilthead seabream juveniles.

Acknowledgements

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FIRST EVALUATION OF ASSOCIATED GUT MICROBIOTA IN WILD THICK-LIPPED GREY MULLETS (*Chelon labrosus*, RISSO 1827)

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Introduction

The need for species diversification in Mediterranean aquaculture has promoted the culture of omnivorous and herbivorous species. Within this context, it is worth noting the high potential presented by the species of the family Mugilidae (mullets), which is considered a priority within the current strategies of European aquaculture (Alexander et al. 2015). One of the mullets with potential for use in aquaculture is the thick-lipped grey mullet (*Chelon labrosus*), due to its great adaptability to different culture conditions, omnivorous profile, high osmoregulatory capacity, and resistance to environmental variations (Pujante et al., 2018). However, there is a lack of information regarding the composition and functions of its intestinal microbiota.

Documenting the bacteria present in healthy individuals is the first step to understanding the impacts of microbial manipulation in aquaculture systems (Tarnecki et al., 2016). In this sense, the knowledge of bacterial diversity from healthy wild fish in their natural environment is essential. Furthermore, the study of the functionality of the intestinal microbiota of wild fish provides information on its capacity to adapt to different culture conditions. Thus, this study aimed to characterize the intestinal microbiota of wild *C. labrosus* and explore its potential functionality on the host.

Material and methods

Eight wild thick-lipped grey mullets (average weight and length: 115.3 ± 5.0 g and 20.3 ± 0.6 cm, respectively) were caught in May 2021 with a net in the Port of Velez Malaga, (36°44’54.9”N 4°03’57.6”W, Malaga, Spain), kept alive and transported to the Centro de Experimentación de Ecología y Microbiología de Sistemas Acuáticos Controlados Grice-Hutchinson (CEMSAC) of the University of Malaga facilities within one hour. Animals were euthanized by immersion in water with a 2-phenoxyethanol overdose (1 mL L^{-1}). Specimens were then individually weighed, and intestinal samples were extracted. Then, each tract was divided into two major sections, anterior and posterior sections, and kept at −80 °C until the subsequent analysis.

Intestinal contents of anterior and posterior sections from wild fish were collected, and DNA was extracted. Subsequently, the V3-V4 regions of 16S rRNA were sequenced using the Illumina technology and results were analyzed by bioinformatics pipeline. The functional profile of the microbial community was analyzed using PICRUSt software.

Results and discussion

Shannon and Simpson diversity indices were significantly higher in the posterior section of wild specimens. The overall taxonomic composition suggests certain homogeneity in the anterior section of the intestine and heterogeneity in the posterior section (Figure 1). Due to this, no statistical differences were detected at any level among both intestinal sections. The intestinal microbiota of wild *C. labrosus* was dominated by the *phylum* Proteobacteria, whereas Tenericutes, Spirochaetes, and Cyanobacteria appeared in the posterior section with low relative abundance. This is consistent with previous studies on wild fish (Liu et al., 2016; Ramírez and Romero, 2017).

Predicted functions of intestinal microbiota showed the most abundant those related to amino acid metabolism, carbohydrate metabolism, energy metabolism, membrane transport, and cell replication and repair. Furthermore, the analysis revealed microbial functional genes related to the elimination of environmental toxins. These functions of intestinal microbiota might provide beneficial effects for the host.

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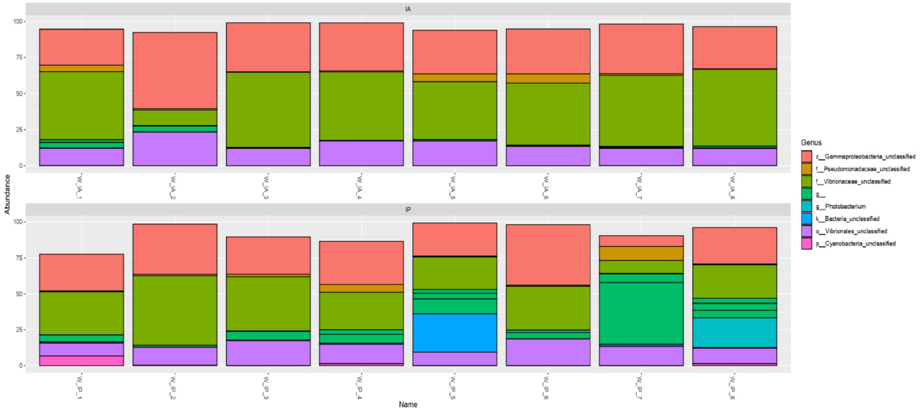

Figure 1. Relative abundance (percentage) of the top 10 genus level for each sample of intestinal microbiota from anterior and posterior section of wild *C. labrosus*. In the figure, W_IA corresponds to the anterior intestinal section of wild fish, and W_IP corresponds to the posterior intestinal section of wild fish.

Acknowledgments

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POTENTIAL POSTBIOTIC ACTIVITIES OF EXTRACELLULAR PRODUCTS OF PROBIOTIC BACTERIA FROM GILTHEAD SEABREAM GASTROINTESTINAL TRACT

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Introduction

Probiotics have been established as a potential tool for improving gut health and environmental quality in aquaculture. However, despite the proven health benefits of probiotics, recent evidence suggests that bacterial viability is not necessary to attain the beneficial-promoting effects (Ang et al. 2020). In this way, postbiotics have emerged providing a potential opportunity in the field of functional foods. They are soluble factors resulting from the metabolic activity of a probiotic or any released molecule, including short-chain fatty acids, enzymes, extracellular products, endo- and exo-polysaccharides, organic acids, etc. that can have interesting properties related to hydrolytic and antagonistic capabilities inducing biological responses on health similar to probiotics while avoiding the necessary administration of live microorganisms (Balthazar et al., 2022). Interestingly, its production can be affected by different factors such as the components of the culture media (Chang et al., 2021) among others. Information on this type of postbiotic activities is very scarce, especially in the case of aquaculture (Mora-Sánchez et al., 2020), so the evaluation of the nutraceutical use of postbiotics to improve health management in fish and other cultivated aquatic organisms is an emerging area of research in aquaculture.

In a previous work, we characterized four potential probiotics (UMA 140, UMA 143, UMA 169 and UMA 216) that were isolated from the gastrointestinal tract of *Sparus aurata* specimens fed with a diet containing a blend of microalgae. This diet involves a selection pressure on the intestinal microbiota of the fish that will be used to achieve enrichment in bacteria with a set of extracellular enzymatic activities capable of metabolizing and mobilizing the components of the diet enriched with microalgae. Here, we investigate the postbiotic potential of the extracellular products (ECPs) obtained from the four candidate probiotics grown on different microalgae-supplemented medium, and evaluate its enzymatic and antibacterial activity, and its cytotoxicity against the SAF-1 cell line. Our objective is to select different ECPs with a variety of activities that help the digestive process of seabream, with the aim to be included in aquafeeds.

Material and methods

The four strains (UMA 140, UMA 143, UMA 169 and UMA 216) were grown on tryptic soy agar supplemented with NaCl (1.5 %) (TSAs) at 23º C for 24 h. Then, one to two colonies of each strain were cultured on 50 mL of tryptic soy broth supplemented with NaCl (1.5%) (TSBs) at 23ºC for 36h (10^9 UFC mL^{-1}, start of the stationary phase) on shaking at 80 rpm. Then, 1 mL of each culture was spread on solid medium plates under the following conditions: i) TSAs (control medium); and solid medium (1.5% agar) supplemented with ii) 5% *Spirulina*; (iii) 5% *Chlorella*; (iv) 5% *Nannochloropsis*; and v) 5% mix of 25% of each algae (*Spirulina*, *Chlorella*, *Nannochloropsis* and *Isochrysis*). *Isochrysis* was not included as a condition because inhibited the culture growth of the four strains. Plates were incubated at 23ºC for 24h and ECPs were obtained by the technique described by Liu (1957). The collection was carried out by adding 2 mL of sterile saline phosphate buffer (PBS). The obtained suspension was centrifuged (10,000 xg, 20 min, 4ºC) and the supernatant was filtered through membranes (0.22μm, pore diameter), and kept at -80ºC until use.

The ECPs were screened for different enzymatic and antibacterial activities. Proteolytic, collagenolytic, lipolytic and amylolytic activities were assayed according to Chabrillon et al. (2005). Phytase, tannase and cellulose hydrolysis were assayed according to Kumar et al. (2010). Antibacterial activity of the ECPs against fish pathogenic bacterial strains *Vibrio harveyi, P. damselae* subsp. *piscicida*, and *Tenacibaculum maritimum* was performed using the agar-well diffusion assay as described by García-Márquez et al. (2021). The hemolytic activity of the ECPs was tested on blood agar plates. In all cases, 50 μL of ECPs were inoculated into 6 mm-diameter wells made in the plates and incubated at 23ºC for 24-48h. The plates were observed for the presence of a clear zone around the wells. Finally, the absence of cytotoxicity of the ECPs on fibroblast SAF-1 cells from marine gilthead seambream was verified, exposing the cells to different doses of the ECPs. After exposure, the viability of the cells was determined by MTT assay according to Espinosa et al. (2018).

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Results and discussion

Only one condition (TSAs 169) was not capable of hydrolyze gelatin, and only two (TSAs 169 and Chlorella 169) did not hydrolyze milk. None of the ECPs had starch, lipase, phytase and tannase hydrolytic activity. Furthermore, only six of the obtained ECPs were capable of hydrolyze the cellulose. In terms of the antibacterial activity of the ECPs, all of them were able to inhibit *P. damselae* subsp. *piscicida*, and only one condition (TSAs 216) inhibited *T. maritimum*.

According to the European Food Safety Authority (EFSA), the evaluation of hemolytic activity is strongly recommended if the isolated bacteria intended to use in food products. In this study, the ECPs obtained from bacterial strains UMA 169 and UMA 216 showed no hemolytic activity. However, ECPs obtained in TSAs medium from strains UMA 140 and UMA 143 showed β-hemolytic activity. Accordingly, due to safety concerns, we discarded from further experiments the ECPs recovered from strains UMA 140 and UMA 143. In view of the results, 5 conditions were selected for further analysis of cytotoxicity: Nanno 169, TSAs 216, Chlorella 216, Nanno 216 and Spirulina 216. The results showed that Spirulina 216 is cytotoxic over the SAF-1 cell line, so its use for addition to feed is not advisable. Further analysis in relation to the contribution of ECP in *in vitro* algae hydrolysis under simulated digestion conditions of seabream enzymes will determine the ECPs candidate with the best potential to be included in aquafeeds.

Acknowledgments

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ANTIVIRAL ACTIVITY OF MEDITERRANEAN AROMATIC PLANTS AGAINST VIRUSES OF INTEREST IN AQUACULTURE

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Introduction

Diseases caused by viruses are one of the main causes of economic losses in the aquaculture sector. It is therefore necessary to search for new antiviral compounds that can mitigate these losses. The aim of this work is to evaluate the antiviral potential of four Mediterranean plant extracts against two viruses of great importance in aquaculture, the viral haemorrhagic septicaemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV). The extracts chosen were *Melissa officinalis*, *Lippia citriodora*, *Cistus salviifolius* and *Rosmarinus officinalis*. These four plants were chosen because their antiviral potential had been previously described.

Materials and Methods

We evaluated the antiviral activity of these extracts *in vitro*, in wo cell lines, EPC (epithelioma papulosum cyprini cell line from Pimephales promelas) susceptible to VHSV infection and CHSE (Oncorhychus tshawytscha embryo cell line) susceptible to IPNV infection, were used for this work. We evaluated the viricidal capacity of the extracts as well as the antiviral activity before or during the course of infection. Viral loads were evaluated by means of qPCR or FFC/mL. Cell fusion inhibition capacity assays were also performed to evaluate the viricidal capacity of the extracts. In addition, the possible immunostimulatory activity of the extracts was also studied.

Results

The results obtained showed that two of the compounds exhibited viricidal and antiviral activity against VHSV, since the treatment with the extracts could diminish the viral loads *in vitro*. The effect of the extracts was observed on the viral particle and before or during the course of the infection. On the other hand, none of the extracts showed viricidal or antiviral activity against IPNV. This work presents promising results for the development of new eco-sustainable antiviral compounds based on plant extracts as therapeutic antiviral treatments in the aquaculture sector.

MOOC “AQUAPONICS – THE CIRCULAR FOOD PRODUCTION SYSTEM“

Zurich University of Applied Sciences, Switzerland

Course description

Aquaponics

Wondering, what fishes have to do with lettuce, kale, basil, tomatoes, aubergine or even vanilla or bananas? Aquaponics is a polyculture that combines aquaculture with hydroponic cultivation, i.e., connects fish farming with soil-independent food production, in a circular system with the aim of recycling nutrients and reusing water from fish farming.

Aquaponic technology continues to attract attention around the globe, especially in the context of urban farming. It is a circular technology requiring a broad knowledge base: from water chemistry, fish welfare, and plant physiology, to engineering and microbiology. Therefore, aquaponics serves also as a learning model for inter- and transdisciplinary thinking and acting.

“*Aquaponics – the circular production system*“ offers a new approach to edX courses. The novelty lies in introducing “real world” experience and skills development into a virtual world of learning. The participants acquire essential knowledge about the Aquaponics, combined with most relevant skills to successfully plan and design a sustainable aquaponic system and ensure the welfare of organisms growing in such as system.

The Aquaponics is the first edX MOOC produced at Zurich University of Applied Sciences ZHAW (Figure 1). It was developed by the Institute of Natural Resource Sciences and supported by the Center for Innovative Teaching and Learning. It is also the first course from any university of applied sciences on edx.org.

Figure 1: Impressions of the aquaponic systems at the Zurich University of Applied Sciences, Institute of Natural Resource Sciences in Wädenswil, Switzerland

Target audience

The MOOC “Aquaponics” is aimed at broad audience with at least an undergraduate level knowledge of the natural sciences (biology, chemistry and physics). However, it is also suited for aquaculture, hydroculture, and aquaponic professionals who wish to deepen their understanding and gain additional insight on specific topics, like for example pest management. The MOOC can be very well included by educators into their own curricula, for example Bachelor’s and Master’s studies.

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Course instructor and experts

The students are guided through the course with introductory videos featuring course instructor Nadine Antenen at the beginning of each new section. The sections of the course are guided by experts (Figure 2) in the field of aquaponics, hydroponics, aquaculture, horticulture, and engineering, who are actively involved in research and teaching at ZHAW.

Nadine Antenen, MSc
Researcher in Aquaponics & Course Instructor

Prof. Dr. Ranka Junge
Ecotechnology Consulting & Expert in Aquaponics

Fridolin Tschudi, MSc
Researcher in Aquaculture (RAS)

Dr. Zala Schmautz
Researcher in Aquaponics & Hydroponics

Dr. Linda Tschirren
Researcher in Aquaculture (fish welfare)

Florentina Gartmann, MSc
Researcher in Aquaponics & Hydroponics (plants cultivation)

Figure 2: MOOC Aquaponics team consists of experts actively involved in the field of aquaponics, hydroponics, aquaculture, horticulture, and engineering.

Course content

The interactive material included in the MOOC (Figure 3) provides instructional scaffolding for the students throughout the learning process. The use of interactive tools and the different edX features combined with a clear thread through the course supports a robust experience for learners. This is probably the reason for the very low dropout rate in the course.

The course content alternates between instructional videos, practical examples and exercises, and excellent visualizations. In addition, a variety of course literature and a course reader in each section support the learner's immersion in the topic. Practical exercises, individual assignments, and quizzes are designed with both, verified and audit students in mind. In the last week of the course, participants also learn how to size and design an aquaponic system.

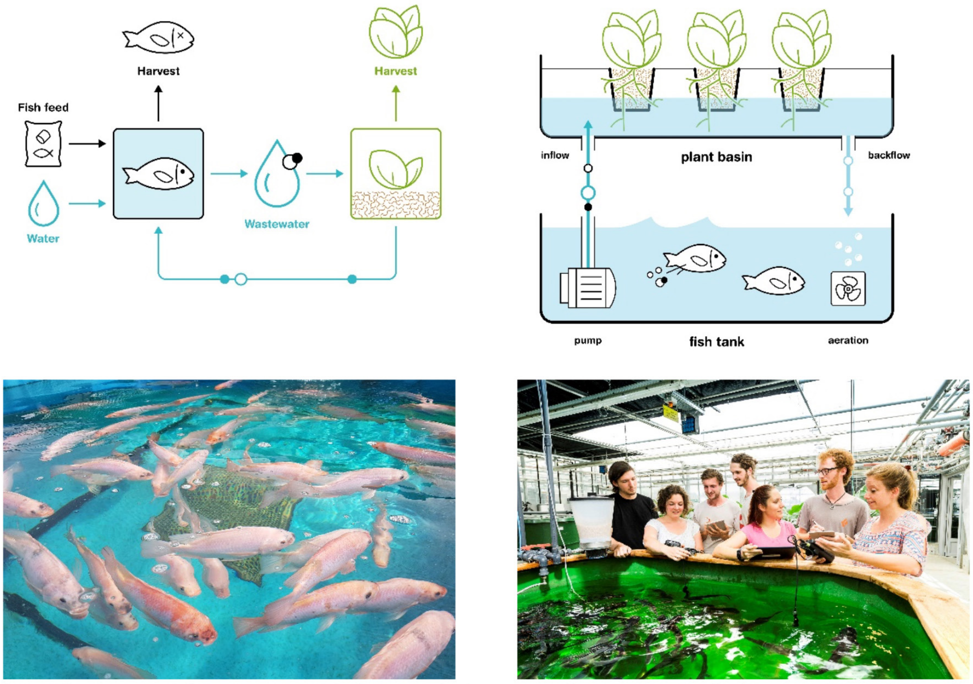

Figure 3: The participants are guided through the course in a very practical way using animated graphics and many photos and videos from our systems.

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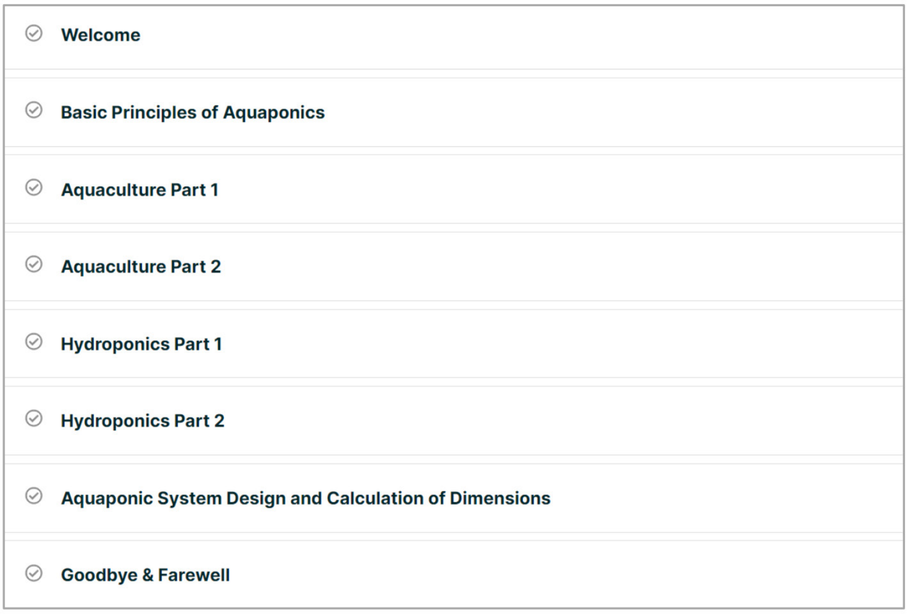
The course offers expert knowledge, gained at ZHAW over more than 20 years, to all, with the most important literature being included in the course catalogue. The students are guided to work independently on a variety of tasks, exchange knowledge in discussion forums and evaluate each other in open-response assignments.

Course structure

The course lasts 6 weeks containing a main topic with a workload of 4-6 hours per week (Figure 4). In the first section, the general aspects of aquaponics are introduced (Basics of Aquaponics; Opportunities, Risks, Challenges, and Limitations of Aquaponics; Aquaponics - A Circular Food Production System). Aquaculture topics are presented in sections 2 (Fish Anatomy and Physiology; Fish Feeding and Growth; Fish Health and Welfare in Aquaculture), and 3 (Recirculating Aquaculture System (RAS) Engineering; RAS Management; RAS Calculations). Hydroponic (soilless cultivation) topics are presented in sections 4 (Hydroponic Systems; Plant Anatomy, Physiology and Growth Requirements; Nutrient Supply) and 5 (Plant Varieties; Monitoring Plants; Integrated Pest Management (IPM)). In its final week, the focus is on implementing what has been learned (System Design and Planning; Calculating the Dimensions of an Aquaponic System) in a practical example (Design and Calculate your own Aquaponic System).



- Welcome
- Basic Principles of Aquaponics
- Aquaculture Part 1
- Aquaculture Part 2
- Hydroponics Part 1
- Hydroponics Part 2
- Aquaponic System Design and Calculation of Dimensions
- Goodbye & Farewell

Figure 4: Course overview consisting of 6 main topics (weeks) with a workload of 4-6 h/week

Each section starts with an introductory video as an overview of that course week. Focus videos address specific topics, such as How to dissect a fish.

The evaluation path (Figure 5) (units marked in pink color) is specifically designed to offer tailor-made evaluation for both, audit and verified learners. Through many interaction possibilities within the different sub-sections, the participants are repeatedly encouraged to become active themselves. This is probably the reason for the high number of active participants.

FIGURE 5 HERE

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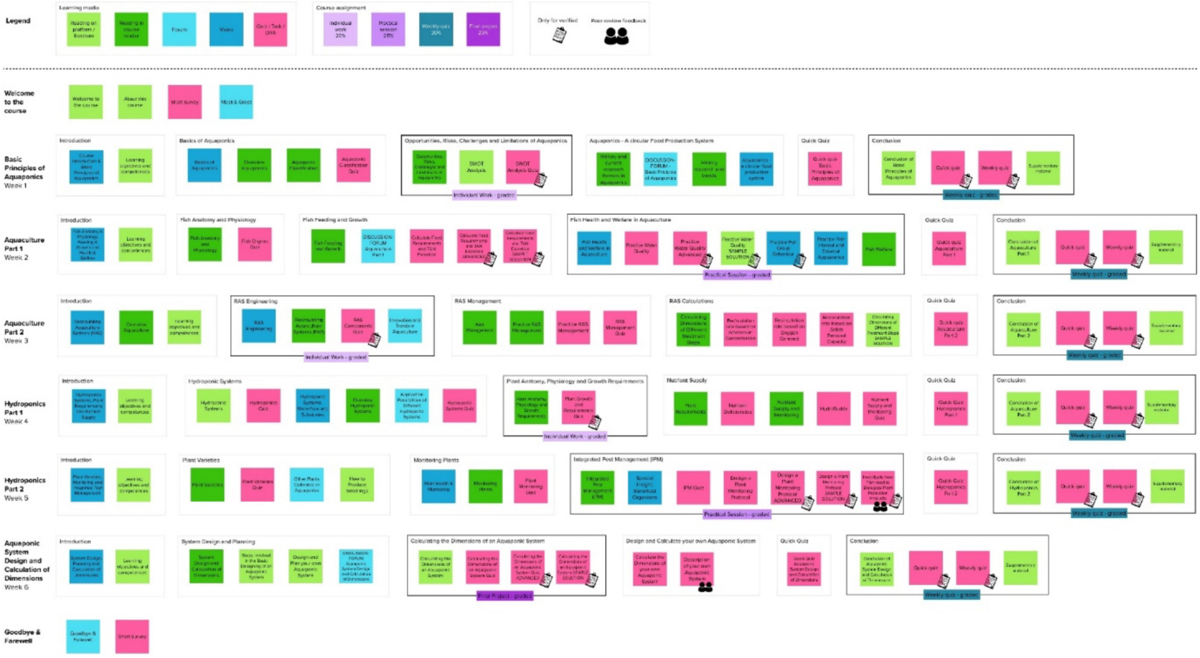
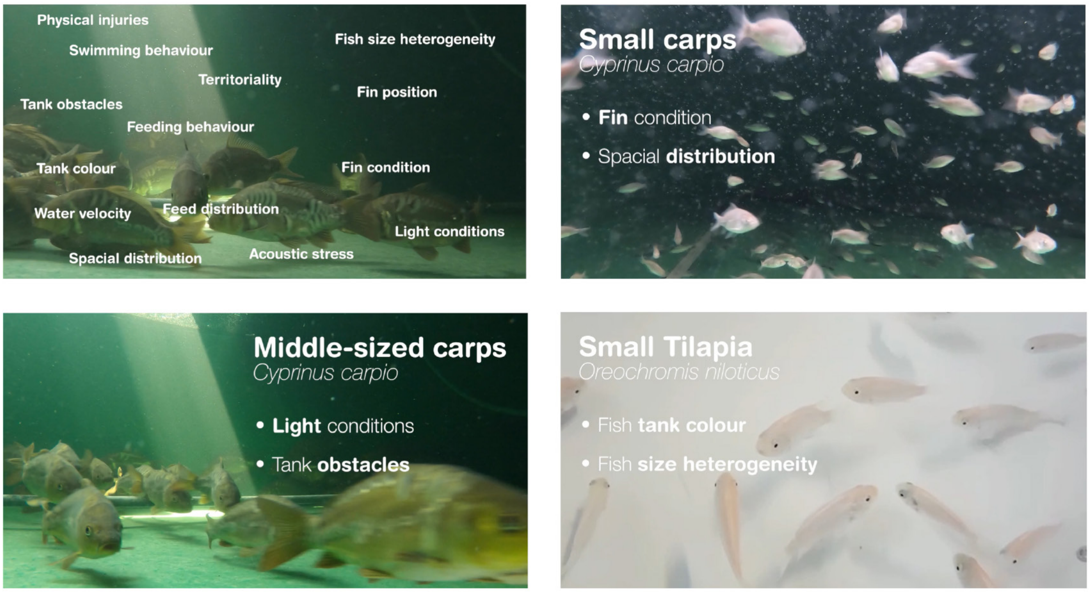


Figure 5: The course structure of MOOC Aquaponics consists of 6 sections. To support the learner's progress different learning media are deployed: Readings on platform/literature (bright green), readings in the course reader (dark green), forums (bright blue), and videos (dark blue). A cleverly done evaluation path (pink) guides both, audit and verified learners, through the course sections and sub-sections.

The practice-oriented learner guidance motivates students to actively participate during the course. Most course materials are available for both audit and verified learners. However, very valuable materials (usually containing the most recent models and examples from our research) are available exclusively to verified students. For example, in the session Practice fish group behavior, underwater video recordings of different fish species in recirculating aquaculture systems are collected. Under the guidance of fish welfare expert Dr. Linda Tschirren, participants (verified track) learn how to observe fish themselves (Figure 6).



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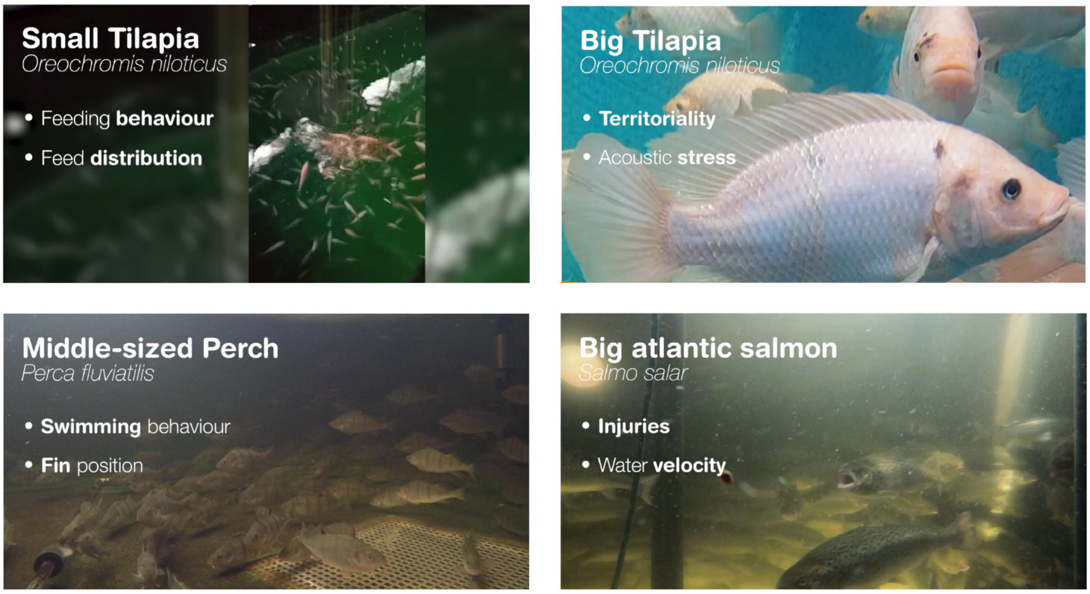

Figure 6: Insights into the practical session Practice fish group behavior guided by a fish welfare expert.

Grading path

This MOOC activates both audits and verified students throughout the course. For this purpose, tasks (individual work, practical sessions, weekly quizzes, final project) are also accessible for audit participants who choose to work only on individual sub-sections. The weekly quizzes consist of a quick quiz (for audit and verified track) and a more detailed final quiz (only for verified track).

In two Open Response Assignments (peer evaluation) on the topics of plant protection in aquaponics and the planning and dimensioning task of their aquaponic system, the participants immediately apply the knowledge they have learned and are encouraged to think further. The participants must gather information, make calculations and be creative by planning a system.

Course support

To ensure adequate support, general discussion forums invite the student to ask for advice on a variety of technical and weekly topics. The participants help each other and where necessary the MOOC team offers support. Several section-specific discussion forums promote the exchange of knowledge among students. For example, in the meet & greet forum, the participants introduce themselves and report about their relation to aquaponics and/or why they attend this course. In another forum, they describe the advantages and disadvantages of different hydroponic systems, and in a task in sections 4 they post about exotic plants which can be grown in aquaponics.

The MOOC team visits the discussion forums several times a week and responds to unresolved, adds clarifications and contributes to discussions. Also, after the first feedback, targeted information material was added to the course to support learners with insufficient knowledge of chemistry.

In addition, during the first instructor-paced course, course communication via email was actively carried out on a weekly basis. In the self-paced course communication is carried out via weekly emails containing the weekly highlights.

Course analytics and interaction

As the first edX course published by the University of Applied Sciences, the MOOC Aquaponics went live in spring 2021 as an instructor-paced course and attracted 2144 participants (5.3 % as verified students) from 128 countries. The re-run 1 in autumn-winter 2021/2022 was self-paced, and 2194 participants were enrolled (5.9 % as verified students). We were pleased to see that the activity of the participants remained high over time (Figure 7).

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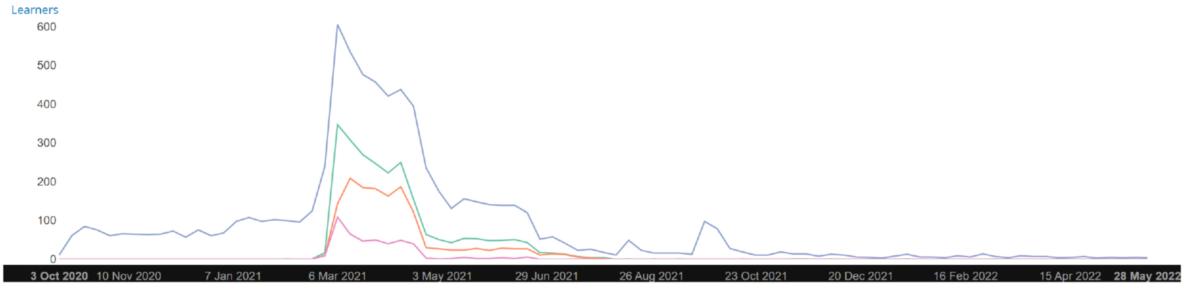
During current course run 929 people (5.4 % as verified students) participant the course.

1st Run instructor-paced



Re-run1 self-paced

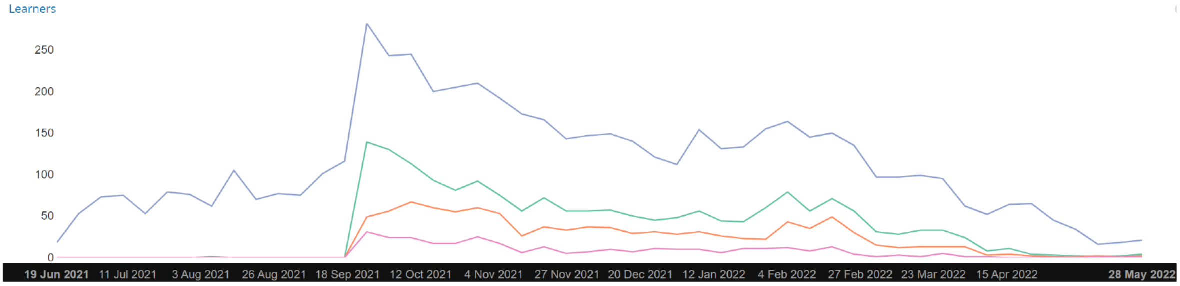


Figure 7: Weekly learner engagement of 1st run as instructor-paced and Re-run 1 as self-paced course.

In the discussion forums, the participants actively exchanged ideas and gathered interesting information. It is really noticeable how the participants are also interested in exchanging knowledge and networking.

Course availability

Because of continuously high interest, this course is now offered twice a year (April-August and October-February) for 5 months each as a self-paced course. In April 2022 re-run 2 has started.

Course feedback

This MOOC is the first one on aquaponics and it attracted very high participation. The reasons for this response are manifold: (i) since 2015, when Aquaponics was proclaimed to be one of the "ten technologies that can change our lives"[1], this topic continues to be relevant in an array of contexts: urban food production, circular economy, research, education, and social work; (ii) The Aquaponics research group at ZHAW is internationally renowned, with several high quality and widely cited papers. It has been found to be the top influential institution in this field in a recently published systematic review[2]; (iii) the Aquaponic community is tightly knit, also due to the COST Action "FA1305 - The EU Aquaponics Hub - Realising Sustainable Integrated Fish and Vegetable Production for the EU"[3], and the word about the high quality of the course spread quickly. Indeed, we received several oral feedbacks from the colleagues from the industry, for example from Michael Reuter aquaponic manufaktur, Sebastien Stoll LinkedIn, Ragnheidur Thorarinsdottir SVINNA-Engineering Ltd., Ulrich Hirschmüller Urban Lab gUG, and Raffael Känzig Phoster GmbH to name just a few.

It is great to see how much positive feedback this course is receiving, which is mostly related to the practical, varied, and open teaching style. The participants enjoyed the diversity and very much appreciated the practical relevance of this course, with contributions of experts and the different, practice-related tasks.

In addition, this course motivates people from different backgrounds to become active in the field of sustainable food production. Many participants reported that they would like to build their aquaponic system in the future or get involved in aquaculture or hydroponics. For example, an airplane pilot started to consider food value chains and interdependencies during the Covid pandemic. After completing the course, he plans to build his own aquaponics farm and produce vegetables and fish for his family.

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Further plans
MOOC «*Aquaponics – the circular food production system*» will be offered twice a year (April-August and October-February). A new MOOC «*Recirculating Aquaculture Systems (RAS)*» will be offered from summer 2023 on. These two courses will be offered as the Professional Certificate «Circular food production systems». In the long term, the MOOC «Hydroponics» should also be offered as a part of this Professional Certificate program.

Learners absolving the professional certificate in circular food production systems will acquire a unique combination of technical engineering skills, biological understanding of the circular systems and the organisms produced as well as the know-how and the inspiration needed to market the end-product. This combination of skills makes the learners fit for the growing markets of urban agriculture, vertical farming, and consulting in aquaponics, aquaculture, and soilless production.

3. Nominee(s) curriculum vitae

Nadine Antenen is a research associate in the Ecotechnology Research Group, where she works towards the water, nutrient, and energy self-sufficiency, with a focus on natural processes in water cycles. She is also an expert in E-Learning.

Florentina Gartmann is a research assistant in the Ecotechnology Research Group. Her expertise is in organic farming, horticulture, vertical farming, integrated pest management as well as sustainable solutions to produce plants and fish in recirculating systems.

Prof. Dr. Ranka Junge (emerita), Institute of Natural Resource Sciences at ZHAW School of Life Sciences and Facility Management. Her expertise and research interest include nature-based technologies involving water and nutrient recycling, such as aquaponics, hydroponics, aquaculture, building-integrated agriculture, and zero-emission buildings.

4. Institutional letter of support

See attachment.

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Plocamium cartilagineum AND *Sargassum vulgare* EXTRACTS MODULATE DIFFERENT SPERMATOZOA TRAITS IN SENEGALESE SOLE

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Introduction:

As the search for novel feed ingredients increases in the aquaculture sector, micro and macroalgae are being widely explored. The three main categories of macroalgae, green, brown and red, already demonstrated to have a diversity of bioactive compounds with considerable prophylactic and treatment potential (Naiel et al., 2021). *Plocamium* and *Sargassum* are two macroalgae genus with proven positive impact on fish health (Carson et al., 2018), mainly due to their antioxidant proprieties (Wang et al., 2019). However, the information regarding the effects of dietary macroalgae in fish reproduction is scarce. In Europe, production of Senegalese sole (*Solea senegalensis*) still faces some constraints in terms of reproduction. The broodstock born and raised in captivity (F1) do not spawn naturally and males present lower sperm quantity and quality (Cabrita et al., 2006), which has been shown to be highly affected by oxidative stress. Our hypothesis is that incorporation of *Plocamium cartilagineum* and *Sargassum vulgare* in fish feeds could confer extra protection against oxidative stress and therefore enhance spermatozoa traits in F1 Senegalese sole males.

Material and Methods:

During the feeding trial, Senegalese sole F1 broodstock was kept under controlled temperature (19 ºC) to ensure the optimal conditions for spermatogenesis. Fish were fed with four different formulated diets for 60 days: Control (Ctrl), Vitamin K1 (VK1), Ctrl supplemented with 5% *Plocamium cartilagineum* (*Pc*), and Ctrl supplemented with 5% *Sargassum vulgare* (*Sv*). Every 15 days fish were sampled to evaluate different parameters of sperm motility, such as total motility (TM), progressive motility (PM), curvilinear velocity (CLV), straight line velocity (SLV) and linearity (LIN), using Computer Assisted Sperm Analysis (CASA) software. Spermatozoa viability and Reactive Oxygen Species (ROS) were assessed by flow cytometer. On the final sampling, 6 fish from each treatment were sacrificed with a lethal dose of anesthesia and gonads were extracted to determine the relative expression of anti- and pro-apoptotic genes (*bcl-2, bcl-x, mcl-1, bad, bax, bok*), and genes involved in fish antioxidant system (*sod, cat, gpx, hsp70*). Specific growth rate (SGR) and feed efficiency were evaluated at the end of the experiment. Statistical analysis was performed on GraphPad 8.0 software.

Results:

Significant differences on sperm quality parameters were evident after one month of trial (2nd sampling) (Figure 1), with *Pc* treatment showing 75 % of spermatozoa TM (A) and the highest viability (B). In turn, *Sv* treatment revealed to have a higher percentage (41 %) of live cells (C). At the end of the trial, males fed with *Sv* diet revealed to have upregulated the gene responsible for the activity of glutathione peroxidase (*gpx)* in gonads (D). Gene expression of anti- and pro-apoptotic genes (*bcl-2, bcl-x, mcl-1, bad, bax, bok*), and genes involved in fish antioxidant system (*sod, cat, hsp70*), was not influenced by dietary supplementation with VK1 or dry algae, as well as SGR and feed efficiency.

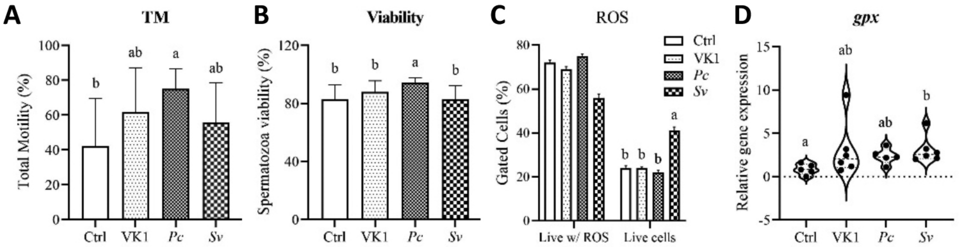

Figure 1. Results from 2nd sampling of feeding trial. A) spermatozoa total motility, B) cell viability, and C) Reactive Oxygen Species are expressed as mean ± SD, and D) gpx gene expression, is relative to the housekeeping genes ubiquitin and elongation factor1a. Differences between treatments were significant when p<0.05. Different letters show significant differences using 1-way ANOVA followed by non-parametric Kruskal-Wallis comparisons (A), (D); and Tukey post-hoc test (B), (C).

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Discussion:

In this study, we have considered four different diets to analyze their antioxidant potential effect on reproductive performance of Senegalese sole F1 males. The VK1 diet was used due to the known effect of vitamin K in many physiological roles, including fish reproduction by increasing testosterone levels (Fernandez et al., 2019). However, the most interesting results obtained here were with the macroalgae supplemented diets. *Plocamium* seems to improve the reproductive performance of spermatozoa, while *Sargassum* upregulated genes involved in gonadal antioxidant system. European seabass fed with *Gracilaria sp*. extracts also revealed higher antioxidant capacity (Peixoto et al., 2019). Further research is needed to uncover the macroalgae bioactive compounds capable of modulating fish reproductive traits and antioxidant system.

Acknowledgments:

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AQUAPONICS IN CESKE BUDEJOVICE, CZECH REPUBLIC: RESEARCH AND KNOWLEDGE TRANSFER TO FARMERS

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Introduction

Aquaponics, i.e. a synergistic connection of hydroponics and aquaculture, is a young industry in the Czech Republic. The prominent research infrastructure in the country is the Aquaponic hall of the Faculty of Fisheries and Protection of Waters, University of South Bohemia. The Aquaponic hall comprises six recirculating aquaculture systems (5 m^3 each), six big-scale mineralization units (1 m^3 each), and four types of hydroponic systems (DWC, Media bed, Drip, Aeroponics) with at least six repetitions. The research group is mainly focused on improving the nutrient efficiency of aquaponics through the development of tailored aquaponics feed, integrated pest and diseases management in aquaponics, effective aquaculture sludge valorization, and knowledge transfer to aquaponic adopters. Further research is focused on changes in the amino acid, fatty acid, and sensory characteristics of fish fed with alternative sustainable feedstuffs.

Research fields

For improvement of nutrient efficiency through the development of tailored aquaponic feed, we used a sophisticated multistep approach based on:

a) Modeling the nutrient efflux from aquafeed with different ingredients with a developed inventory entitled Tilafeed (Roy et al., 2022),
b) Making the aquafeed in-house with the extruding machine,
c) In-house digestibility and nutrient retention and losses studies in the Guelph system (Fig. 1.; Roy and Mráz, 2021),
d) Applied studies in an aquaponic system with mineralization of sludge.

Integrated pest and disease management and its bottlenecks in aquaponics were reviewed (Folorunso et al., 2021). Further experiments are focused on the runoff of pesticides into aquaponic water and its effects on fish and nitrifiers in the biofilter. Moreover, since numerous biocontrol agents are commercially available against insect pests, further research aims to develop novel bioagents against common fungal diseases, which are hard to tackle with natural fungicides. These bioagents include several entomopathogenic fungi, which are first tested *in vitro* (Fig.2.). The best candidate is then tested *in situ*.

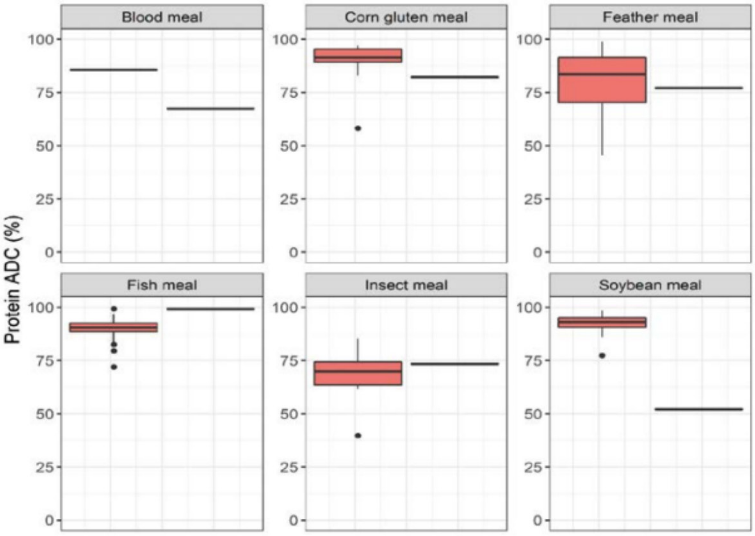

Fig. 1. Comparison of digestibility of protein sources using the in-house technology (black line) with global metadata on the same protein feedstuffs (pink boxplot) for tilapia.

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Fig. 2. Efficacy of a novel bioagent against powdery mildew on cucumber leaves. (A) leaves treated with 0.05% tween 80 solution, and (B) leaves treated with the novel bioagent.

We use several approaches for valorizing aquaculture sludge, including aerobic digestion in continuous big-scale mineralization units or lab-scale batch reactors, vermicomposting aquaculture sludge (Kouba et al., 2018), and/or/or biofloc technology.

Knowledge transfer to aquaponic adopters is based on regular meetings with aquaponic farmers. As most of the aquaponic farms in the Czech Republic were installed by the same company, they all suffer from the same flaws, i.e. undersized water and air pumps and mechanical and biological filters. With the help of national funding agencies, the technologies of two of the farms were already improved, and these farms are profitable.

Since the sustainable feedstuffs in aquafeed can substantially alter the composition of fish flesh, the final product is analyzed for amino acids, fatty acids, and sensory characteristics by certified panelists.

Acknowledgment
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TOWARDS A SUSTAINABLE AQUAPONIC FARM MODEL IN BELGIUM: PRODUCTIVITY AND ECONOMIC ANALYSIS OF TWO CONTRASTING FISH PRODUCTION MODELS (TILAPIA VS PIKEPERCH)

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Thanks to its key strengths such as water use efficiency and nutrient recycling, aquaponics is considered as a sustainable food production method and is still getting growing interest in the scientific community, producers and a broad general public. It meets commonly shared consumer expectations toward food production such as: local production, fresh, healthy (pesticide-free), diversified products, environmentally friendly and low CO_2 emissions. It is also in line with the EU strategy to promote sustainable food systems, specially fish farming. However, commercial aquaponics production remains in its infancy as regulations and profitability weakness act as a serious brake on further growth (Turnsek et al., 2020; Fruscella et al., 2021).

With the aim of creating robust foundations for the development of an aquaponic farm in Belgium, we set up an aquaponic pilot production system designed to test and compare different agronomic and economic models, sizeable to a commercial scale.

This coupled aquaponic system (total volume: 18 m^3) presents a 8 m^3 fish production capacity and 50 m^2 of crop production in raft and NFT, equipped with LED lighting. The two fish production models were tilapia (*Oreochromis niloticus*), a tropical fish species, highly productive, with a relatively low market price, and pikeperch (*Sander lucioperca*), a temperate fish, less productive but with an added commercial value. Vegetable production included lettuce, basil, coriander, parsley and rocket salad.

Data covering biological production (growth and survival), energy and water use, and physico-chemical functioning were collected during 1 year for tilapia and during 2.5 years for pikeperch production, supporting a deep analysis of fish-vegetable productivity, and operational costs.

A market analysis targeting distribution of aquaponic products was also carried out to quantify the local demand and sale prices, define the distribution channels and their capacity. First results show that, even if tilapia is 3-fold more productive than pikeperch, associated vegetable production was relatively higher with pikeperch and economical balances are similar for both models, as the high value of pikeperch offsets its lower production.

Finally, production, economical (investment and operational costs) and market data will be evaluated together to come up with recommendations on operational marketing (targeted products and markets) and more technically, on farm sizing and design. The ultimate output will be the production of a business model integrating all these data and proposing a reliable aquaponic farm model.

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TRACKING AND ANALYSIS OF THE SWIMMING ACTIVITY OF EUROPEAN SEABASS *Dicentrarchus larbax* IN SEA CAGES AND ITS RELATION TO FEEDING

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Introduction

Feeding is the primary factor determining efficiency and cost in aquaculture and requires optimization to decrease feed loss and maintain fish health. Monitoring of individual and group swimming patterns in response to external factors such as feeding can provide useful information for improving production management. For this reason, intelligent feeding control based on the detection of behavioral changes and growth status has gained increasing attention and efforts are being made to define behavioral indicators to detect satiation levels and control feeding for different species. Here, an automated routine that enables individual fish tracking in sea cages as well as the detection of different swimming patterns is developed. Its potential applications on the detection of the swimming behaviour around feeding times are also presented.

Materials and methods

A group of E. seabass fish of 200 g body weight was reared in a circular polyester cage (40 m diameter, 9 m depth) located at the pilot scale netpen cage farm of HCMR at Souda bay, Crete (certified as an aquaculture facility from the national veterinary authority; code GR94FISH0001). A submerged network camera (Fyssalis V3.1) capturing at 10 fps was used for monitoring and video recording during daylight hours in April 2022. The camera was positioned at 4 m depth using a gyroscopic gimbal stabilizer to ensure it pointed upwards. Manual feeding was performed once daily, between 08:00 to 10:00. A machine learning model for tracking people (DeepSort) was trained and adapted to track fish individually (using OPENCV/Python) and extract their speed and direction. In addition, computer vision techniques that can detect feeding events and group polarized movement were incorporated into the model.

Results

The system is capable of distinguishing between three different movement patterns related to feeding (figure 1). The polarized motion seen before feeding is realized (figure 1 left), motion that resembles feeding behavior but lasts for a limited time period and could be an indicator of feeding or other warning situation (figure 1 middle) and the feeding event where the fish shoal swirls around the feed (figure 1 right).

The system is also capable to detect variations in the activity of the fish. Extraction of the group speed for two consecutive days showed significant variations of the fish speed during the day, suggesting that the fish start being more active during and after feeding and less active in the afternoon. More specifically, the group's speed increased from 0.2 bd/s to 1.1 bd/s during the feeding window (i.e. from 08:00 to 10:00, figure 2), and it remained at maximum levels until 12:00, two hours after feeding. Then it started decreasing until it reached a minimum speed of 0.2 bd/s at midnight. This pattern is repeated also in the second day.

Conclusion

The presented system can successfully capture speed variations and different swimming patterns related to feeding or other external factors and can be used to provide useful information on the dynamics of the movements and possible critical values that indicate a transition from hungry to satiated states resulting in the better control of the feeding process. Changes in speed can successfully be captured, suggesting that fish start increasing their activity just before feeding something that would indicate anticipation for feeding and decrease their activity in the afternoon. Further studies are required to help us understand the contribution of other factors such as the human presence, or the internal circadian rhythm on the variation of the activity.

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Figure 1. Automatic detection of three different movement states of the group that are related to feeding, i) polarized motion seen before feeding (left), ii) swirling motion of limited time duration (middle) and typical swirling during feeding (right).

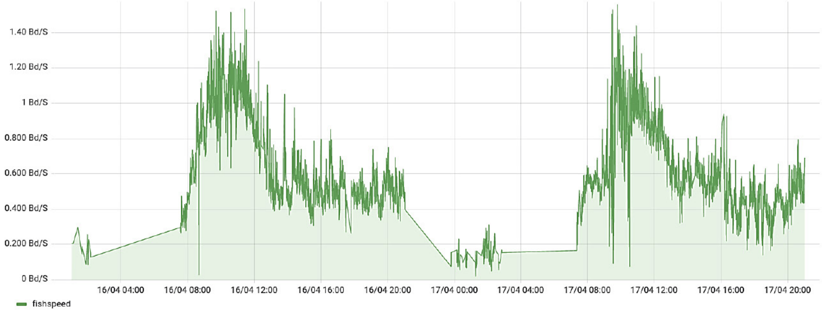

Figure 2. Variations in speed for two consecutive days in April 2022.

Acknowledgments

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HOW COMMON IS COMMON? EXPERIENCES AND RESULTS DERIVED FROM RESTORATION PROGRAMS OF THREE STURGEON SPECIES: *Acipenser naccarii, A. oxyrinchus*, AND *A. sturio*

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Introduction

Sturgeons in Eurasia are under increased pressure of extirpation and, as a result *ex situ* measures including the safeguarding of remaining genetic heterogeneity and supporting the recovery of the populations in the wild, are essential.

Since it is widely accepted that the targets and rearing practices between commercial rearing and conservation rearing do differ substantially in terms of fitness, this presentation attempts to describe those practices using three study cases in comparison with commercial sturgeon aquaculture. The Adriatic sturgeon (*A. naccarii), the American or Baltic sturgeon (A. oxyrinchus),* and the European sturgeon *(A. sturio*) all are critically endangered and face the threat of (local extinction) in the recent past. The three species currently are subject to regional restoration programs that differ substantially with regard to the organization of the recovery projects.

Materials and Methods

The analysis will summarize and compare the approaches taken, experiences made and the results obtained in an attempt to emphasize practicable yet safe results for the restoration programs. The analysis comprises not only rearing practices but also looks more deeply into the administrative background, the strategic approaches taken, the solutions for establishing *ex situ* stocks as well as rearing and release programs, funding and regional integration.

Results and Discussion

Since Adriatic and European sturgeon recovery programs were established in the 1980s based on the remaining populations of the species in the Po and Gironde rivers, the prerequisites differed from the reintroduction of the Baltic sturgeon that was extirpated when the project came to life. All three projects established captive (*ex situ*) broodstocks in an attempt to save the species from extinction and to provide a basis for the subsequent reproduction and release of the offspring. The comparison addresses the strategic and administrative background of the projects, analyzing the involvement of governmental agencies in funding and in accompanying the process of the recovery/reintroduction.

The means of broodstock development, reproduction and rearing are investigated and the practices of the releases are compared from the technical as well as from the administrative point of view. The strengths and weaknesses of the projects are illustrated and discussed.

As such, the presentation aims at providing a baseline for the planned follow up discussion to identify the options for a closer collaboration between commercial farms and recovery projects. The essential prerequisites, the benefits and the drawbacks in the framework of such a strategic collaboration as well as the possible options for its implementation will be assessed.

ENVIRONMENTAL QUALITY IMPROVEMENT AFTER THE CONVERSION OF A MARICULTURE PLANT INTO AN IMTA SYSTEM

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Introduction

Mariculture activities strongly affect the surrounding environment (Gentry *et al.,* 2017). Wastes deriving from these activities are the main cause of negative environmental impacts influencing water quality and benthic communities (Wang *et al.,* 2020). Within the project Remedia life, a fish mariculture plant (Maricoltura Mar Grande of Taranto) was converted into an Integrated Multi-Trophic Aquaculture system (IMTA), utilizing a new set of very efficient bioremediating organisms (polychaetes, porifers, mussels and macroalgae). To investigate the restoration effect occurring after the conversion of the plant, an environmental monitoring program was performed at the beginning of the project (Giangrande et al., 2022) and repeated after two years of the IMTA system establishment. In order to obtain a detailed description of the environment, both water column and sediment were sampled.

Material and methods

The study area is located on the south-west side of the Mar Grande of Taranto (40°25′56″ N; 17°14′19″ E) (Ionian Sea), which is a semi-enclosed basin connected to the Gulf of Taranto. The investigation was performed in the aquaculture plant "Maricoltura del Mar Grande (MMG)", partner of the Remedia-Life project. Two sampling areas were identified around the fish farm, with 4 stations located at the four corners of the plant. Samples were collected one year before (2018) and two years after the realization of the IMTA system. Physical-chemical and microbiological parameters and TRIX index were measured in the water, whilst microbiological parameters and macrobenthic analysis were conducted in the sedimentary environment, considering the AZTI's Marine Biotic Index (AMBI) and microtox bioassay. Moreover, the changes in biodiversity of hard substrates present under the cages were also investigated.

Results

The ex ante analysis allowed to asses the more impacted station due to the aquaculture activities. By comparison, the other three stations were characterized by a higher environmental quality, therefore the more impacted station was chosen to experiment the bioremediating activity and considered as the Treatment station (T). Another station, without bioremediators, was utilized as Control (C). An amelioration of the Treatment station was observed after the conversion of the plant. By contrast the Control station remained quite similar throughout the years, leading to conclude that the bioremediating activity determined a similarity of the environmental condition in the investigated stations, towards an amelioration of T station at the end of the project. The changes observed over time in T station of both water column and sediment parameters are reported in the following figure (Fig. 1).

Discussion

In the realized IMTA system the bioremediating organisms grew without adding any feed other than the fish one. The synergistic action of all the bioremediators reared around the fish cages restored the water column by transforming the waste into biomass that can be utilized as a by-product of aquaculture. More in particular, macroalgae avoid the eutrophication of the water surrounding the fish cages, whilst the filter feeders, such as worms, sponges and molluscs remove bacteria and particulate organic matter, improving the microbiological quality of the water as well as the transparency. Our results indicate that the marine environment benefits from the eco-sustainable potential of this method. The values of the parameters selected as indicators of environmental quality, such as TRIX index, AMBI microtox bioassay, microbiological analyses and hard bottom biodiversity evaluation, have significantly showed an amelioration of the Treatment site. This was particularly true for the indicators related to the sediment compartment which represents the biological memory of the system. In fact, the environmental quality under the cages, once highly degraded, was recovered with a remarkable increase in local biodiversity. Our results are noteworthy since for the first time the bioremediation effect was *in situ* measured.

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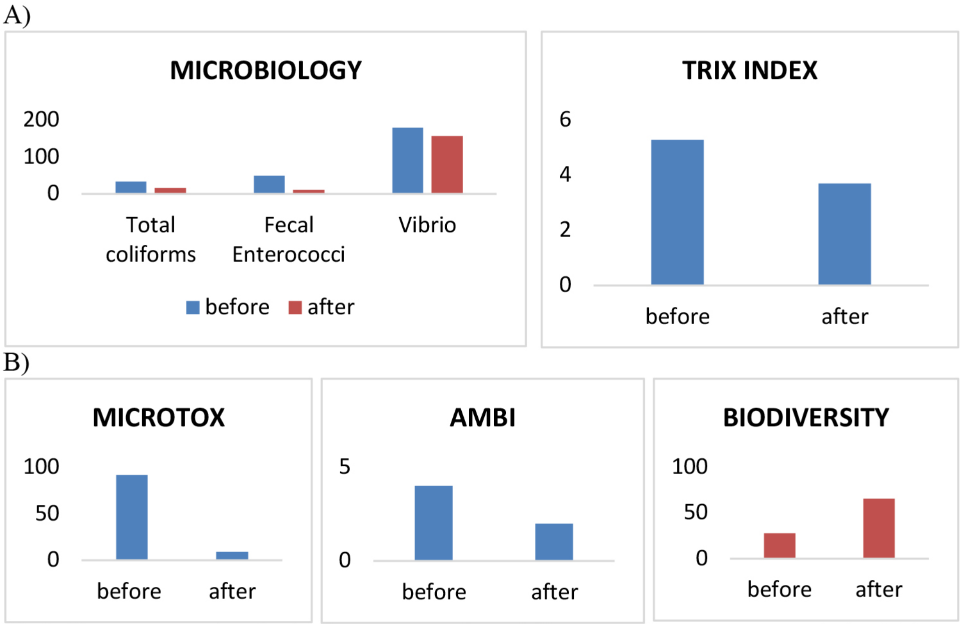

Fig.1. Changes over time in the Treatment station of A) water column parameters (microbiology analysis and TRIX index) and of B) sediment, soft and hard bottom parameters (Microtox, AMBI and biodiversity).

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HISTOPATHOLOGICAL DIFFERENCIAL DIANGNOSIS OF BACTERIA-RELATED GRANULOMAS IN NILE TILAPIA, *Oreochromis niloticus*

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Introduction

Intensive tilapia culture is often affected by a wide range of bacterial infections, some of them exhibiting similar clinical presentations that are difficult to differentiate even for the most trained eye. In these cases, histological techniques presents a valuable diagnostic tool, not only to detect infectious and parasitic agents, but also to understand the clinical context, the pathogenesis and to identify possible concomitant agents sometimes difficult to detect by other diagnostic techniques. This work summarises findings and diagnostic criteria of granulomatous lesions associated to different bacterial agents.

Material and methods

Symptomatic fish were sampled, necropsied and organs were immediately fixed in 10% buffered neutral formalin on site during farm visits. Fixed organs were embedded in paraffin in our laboratory. 4 μm-thick sections were performed, mounted on glass slides and stained using Haematoxylin & Eosin (H&E) standard protocols. Additional histochemical staining techniques including Gram, Giemsa and Ziehl Neelsen were also used to better identify bacterial agents.

Results and conclusions

Macroscopical examination of internal organs revealed multiple whitish granulomas. These lesions were especially prevalent in kidney and spleen.

Histological examination presented with variable impact of granulomatous reaction including:

Focal necrosis and macrophages aggregates containing intracellular, long rod-shaped, gram-negative bacteria were detected (Fig. 1) consistent with *Francisella sp*.

Well-defined granulomas, with a thick peripherical layer of epithelioid cells and a core formed by abundant cellular debris and high counts of free and phagocyted gram-negative cocci bacillary bacteria (Fig. 2) consistent with *Edwardsiella sp*.

Well-defined granulomas, with a thin fibrous capsule, high numbers of melanised macrophages and high counts of acid-fast stained fine bacilli (Fig 3) consistent with *Mycobacterium sp*.

Given the similarity of lesions, routine H&E-stained sections and additional use of histochemical stains allow us to fully characterise the lesional pattern and and provide a morphological diagnosis. Obtained results also help to stablish a diagnostic approach, especially in those cases where microbiological analysis fails in its attempts or theis is not available. Specific techniques, such as immunohistochemistry or polymerase chain reaction are often necessary for confirmatory diagnosis.

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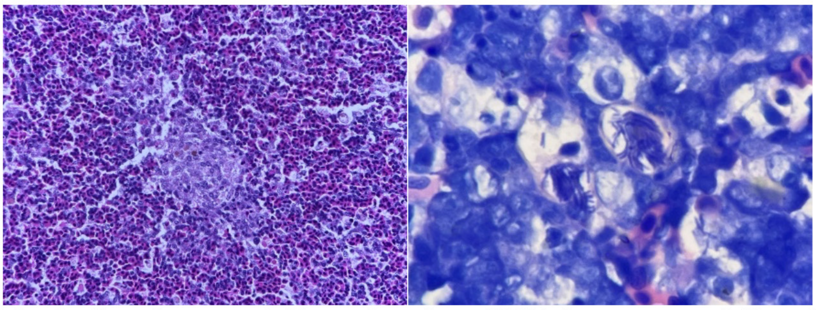

Fig. 1. Granulomatous infiltrate in spleen (H&E, low power – left) and macrophages with multiple phagocytized long rod-shaped bacilli (Giemsa, high power – right).

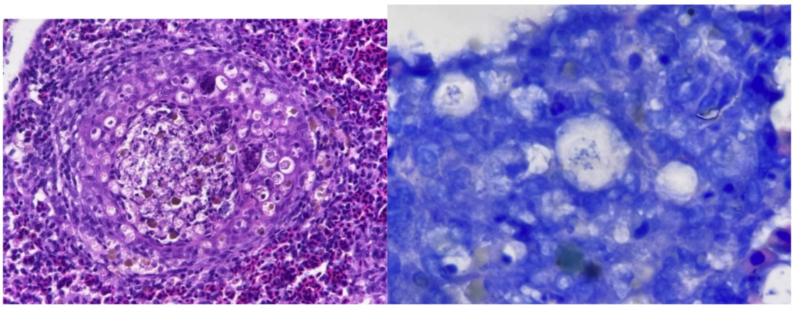

Fig. 2 – Granuloma in spleen (H&E, low power – left) and macrophages with multiple phagocytized cocci-bacilli (Giemsa, high power – right).

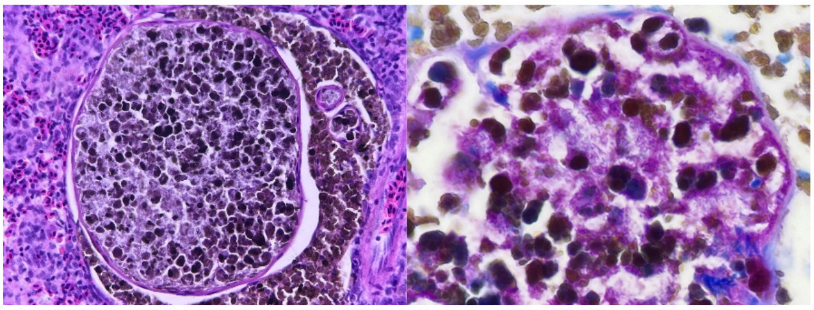

Fig. 3: thin-walled granuloma (H&E, low power -left) and high counts of acid-fast stained free bacilli within granuloma (Ziehl Neelsen, high power-right)

CORRELATION BETWEEN POPS ABSORBED BY BEACHED RESIN PELLET AND THEIR DEGRADATION CONDITIONS IN SEVERAL ITALIAN SITES: A COMPARISON BETWEEN “MUSSLE WATCH” AND “PELLETS WATCH” MONITORING

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Introduction

Nowadays, coastal pollution monitoring consists in study environmental media, such as sediments, water or/and biological samples, as mussels. A new challenge started in 2009 with the International Pellet Watch (IPW), proposing the use of resin pellet as no-living passive samplers, in order to avoid long and high-cost preparation samples of coastal environmental media. Positive correlation (Fig. 1) between POPs concentration in mussels and in resin pellet has been proved during IPW [1].

Resin pellets are the small virgin plastic particles of different polymeric types from which all plastic objects are made. During plastic production processes, industries and factories disperse resin pellets in the environment and most of them arrive in the ocean standing there for a lot of time. Due to their specific weight almost the same of salt water, many of types of pellet floats in the marine microlayer, and many of them and many of them end up on the beaches.

As the main POPs (persistent organic pollutant) and linear hydrocarbons absorption takes place in the microlayer where pollutants are most concentrated than in the water column. In this way pellets can sorb hydrophobic substance [2], [3].

The environmental pollution mapping methods proposed within the IPW is based on Endo et al. (2005) observation regarding a correlation between the degradation state of resin pellets (especially the PE ones) and their POPs content. They sustain that a more pronounced coloring (yellowing) of the pellet should indicate a longer residence time in the sea of such particles, and therefore a greater degradation of their polymeric matrix; the longer time spent in the sea also means a greater probability of having absorbed pollutants dissolved there, from which should follow a correlation between more intense coloration and POPs penetrated. This means that, by selecting the darkest pellets present at different sites, it is possible to get an idea of the degree of pollution in that particular area [4, [5].

As Endo et al. (2005) considerations are supported by a very small number of sampled pellets, we decided to undertake an “Italian pellets survey” in order to investigate, with a greater sample number and so a major statistical accuracy, the structural characteristics of beached pellets, trying to understand if exists a correlation between physical/chemical degradation state, their color (yellowing) and the organic pollutant concentrations.

Materials and methods

We sampled pellets distributed in all the Italian peninsula during spring/summer of 2019, thanks to the contribution of volunteers of Italian NGO Legambiente. Then, we selected sites presenting a high pellets density (more than 100 pellets for site) and, following the prescription reported in Endo et al. 2005 [1] and Ogata et al 2009 [4], we separate them in 3 “yellowing” classes. So, we investigated their polymeric structure and degradation characteristic with infrared analysis, and finally we proceeded to extract POPs from them.

Polymeric structure was identified with ATR analysis. Spectra were registered using a Fourier Transform-InfraRed Jasco 6200 (Jasco, Tokyo, Japan) equipped with a PIKE MIRacle (Madison, WT, USA) accessory, in the Laboratory of Istituto per i Processi Chimico-Fisici of Consiglio Nazionale delle Ricerche (IPCF-CNR), in Pisa. Each sample underwent 64 scans from 4000 to 650 cm^{-1}, after the collection of background data.

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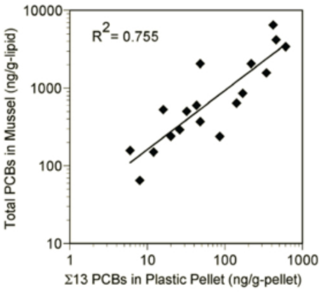

Fig. 1. Correlation between of ΣPCBs in mussels (ng/g-lipid) and in plastic pellets (ng/g-pellet) [1].

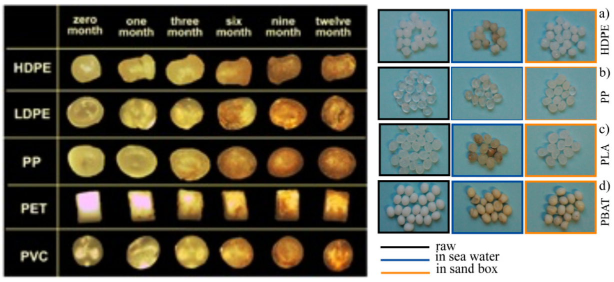

Fig 2. On the left: Discoloration of different polymeric matrix of pellets over time [3]. On the right: Digital photos of the raw pellets of standard of bio-plastic (in black), 6 months-aged in seawater (in blue) and 6-months-aged in sandbox reproducing beach conditions (in orange) [5].

The Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZS PLVA) developed POPs (PCBs and PAHs) extraction and analysis. Sample preparation consisted in a vigorous extraction in n-hexane in ultrasonic bath for 30 minutes at 50°C. No purification step was applied. PCBs analysis was performed in GC-MS/MS ThermoFischer (Waltham, MA, USA) SCIENTIFIC TSQ 8000 Evo equipped with autosampler Thermo SCIENTIFIC AI 1310 hyphenated with spectrometer QqQ Thermo SCIENTIFIC TRACE 1300 with EI ionization mode. The column utilized was a Zebron ZB-SemiVolatiles (30m x 0,25mm x 0,25μm) with a 5m Guardian End Phenomenex and injection temperature has been set at 260°C. Injection was operated in splitless mode with a 2 μL volume. Acquisition was operated in SRM (Selected Reaction Monitoring) mode. As mobile phase was used He at 1.2 mL/min. PAHs analysis was performed in HPLC-FLD 1100 Series quaternary pump (Agilent Technologies, Santa Clara, CA, USA). Data acquisition and analysis were performed using ChemStation® software. Fluorescence $\lambda_{ex}/\lambda_{em}$ was 294/404 nm, gain 12x and injection volume 1 mL. Stop time at 36 min; and column temperature 25 °C. Chromatographic separation was performed using a reversed-phase column Envirosep pp (125m x 4.6mm x 5μm). The eluents were: water (A) and acetonitrile (B). The flow rate was 1 mLmin^{-1}.

Results

At the moment the results are still preliminary and under elaboration, but we hope to have them ready for presentation at the Conference "Aquaculture Europe 2022". In this way we will be able to discuss the validity of this method compared to the more standardized method using mussels as "sentinels" for environmental pollution in coastal areas. Pellet watch could certainly have some advantages (easier sampling and analysis of such inorganic samples), but it must be considered their greater mobility compared to that of mussels, which are sedentary organism. Moreover, although this method has been used for many samplings worldwide, starting from the one proposed by Ogata et al. [4] onwards [5], [6], [7], no accurate assessment has yet been made (supported on a statistical basis greater than that of the work of Endo et al. [1]) to support and corroborate the validity of the supposed correlations between pellet color, degradation status and pollutant content.

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THE ADIPOSE FIN IN SALMONIDS - RUDIMENTARY APPENDAGE OR FLOW SENSOR?

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Introduction

Hundreds of millions of salmonid fish are tagged annually by removing the adipose fin before release, for example for recapture statistics and fishing. Previous experiments showed that the resection of the fin decreased swimming performance, particularly of juvenile fish. Recent research suggests that adipose fin clipping conflicts with animal welfare (Reimchen & Temple, 2004; Templ & Reimchen, 2008; Buckland-Nicks, 2016). The adipose fin is not only a useless body appendage that consists of fat cells. Microscopic analyses of the adipose fin identified a complex network of nerve fibers. Our gene expression studies support the hypothesis of nerve activity in the adipose fin. Our preliminary data together suggest a sensory function of the fin that may allow fish to align optimally with the water flow and swim more efficiently. We therefore began a targeted study to generate data that support the hypotheses of the acute and chronic stress and the potential pain response due to fin resection and furthermore the expected mechanoreceptive function of the adipose fin. As part of a multiparametric approach, we performed comprehensive gene expression analyses, conducted holistic transcriptome studies over time, plotting behavioral responses, and recorded stress hormone activity.

Results

We profiled the specific expression of 20 neuron- and glial cell-marker genes in the adipose fin and seven other tissues (including dorsal and pectoral fin, brain, skin, muscle, head kidney, and liver) of the salmonid species rainbow trout *Oncorhynchus mykiss* and maraena whitefish *Coregonus maraena*. Moreover, we measured the transcript abundance of genes coding for 15 mechanoreceptive channel proteins from a variety of mechanoreceptors known in vertebrates. The overall expression patterns indicate the presence of the entire repertoire of neurons, glial cells and receptor proteins on the RNA level. This quantification suggests that the adipose fin contains considerable amounts of small nerve fibers with unmyelinated or slightly myelinated axons and most likely mechanoreceptive potential. The findings support a previous hypothesis about the innervation and potential flow sensory function of the adipose fin. Moreover, our data suggest that the resection of the adipose fin has a stronger impact on the welfare of salmonid fish than previously assumed (Koll et al., 2020).

Conclusion

Our data suggests that the adipose fin is fully innervated by small nerve fibers with, most likely, mechanoreceptive potential. Neurons, glial cells, and receptor proteins seem to be present on the RNA level. This supports the hypothesis about the adipose fin as a flow sensor (Reimchen & Temple, 2004; Buckland-Nicks, 2016). Our data accelerate the discussion about the use of adipose-fin clipping. The adipose fin is a criterion for the choice of suitable sexual partners (Westley et al., 2008) and, it contributes to the swimming efficiency (Templ & Reimchen, 2008). Thus, the resection of the adipose fin tissue seems to be a less suitable method, particularly from an economic point of view regarding sea ranching and large-scale aquaculture in the future.

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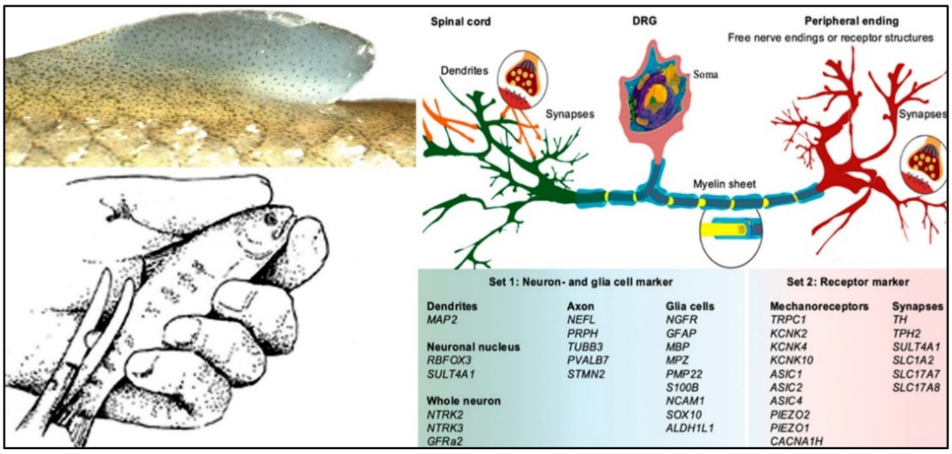

Figure 1. *Adipose fin of juvenile rainbow trout* ***(upper left);*** *Clipping of the adipose fin in Atlantic salmon parr (Mortensen, VKM Report 2016)* ***(down left)****; Candidate genes of the responding nervous system after adipose fin resection* ***(right)****.*

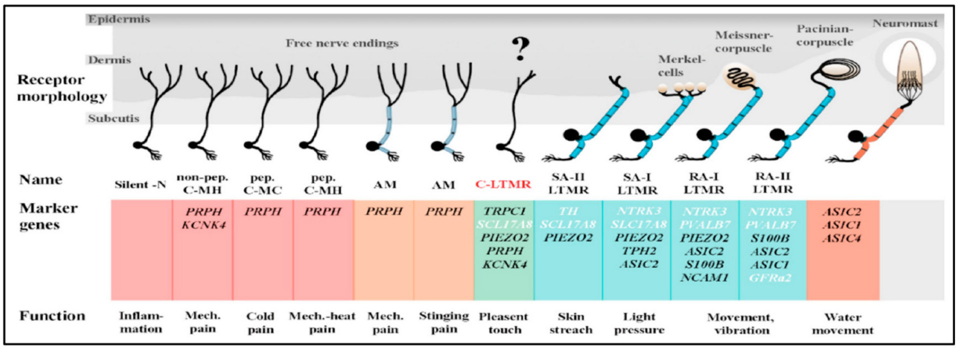

Figure 2. Summary of the possible presence of somatosensory receptors in the adipose fin. Listed are the genes indicating the presence of nociceptors with free nerve endings (**red, orange**) and with specialized receptor structures (**green**) and cutaneous low-threshold mechanoreceptors (LTMRs) with receptor corpuscles (**blue**) as well as neuromasts (**light red**). Marker genes identified in the adipose fin in ample amounts are printed in black. Marker gene names are printed in white if the expression in the AF was not outstandingly high in the comparison of the tissues analyzed.

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DEOXYNIVALENOL AND FUMONISIN B1 IN GILTHEAD SEABREAM: IMPACT ON GROWTHPERFORMANCE,HAEMATOLOGY,IMMUNOLOGY,ANDHISTOPATHOLOGY

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Introduction

The awareness of mycotoxin-related issues in the aquaculture industry has been increasing, accentuated by the increased inclusion levels of plant meals in aquafeeds. Mycotoxins are secondary metabolites produced by fungi, often found in agricultural products, used to feed livestock, and pose a health risk to both livestock and consumers. Fumonisin (FB) and deoxynivalenol (DON) are among the most frequently detected mycotoxins at high levels in fish feed (Gonçalves et al., 2020). These mycotoxins can potentially cause problems in fish farms, with significant economic losses such as mortality, reduced productivity, higher susceptibility to diseases, etc (Koletsi et al., 2021). The effects of different contamination levels of dietary mycotoxins FB and DON on growth factors and health parameters in gilthead seabream (*Sparus aurata*), were presently assessed.

Materials and methods

Gilthead seabreams (3.40±0.5g) were transferred to the Aquaculture Laboratory facilities, University of Thessaly from a commercial fish farm, and randomly placed in 21 glass aquariums (125L, 30 fish/aquarium). The mycotoxins Deoxynivalenol (DON, Cayman Chemical) and Fumonisin B1 (FB, Cayman Chemical) were dietary administered at different contamination levels to the experimental fish. Six experimental diets in triplicate were applied: DON-A (300ppb), DON-B (2000ppb), DON-C (5000ppb), FB-A (5ppb), FB-B (10ppb), and FB-C (40ppb). The control group (CTRL) was fed with mycotoxin-free diet. Water quality parameters were systematically monitored and the water temperature was kept stable at 21 ± 1°C. Fish were fed *ad libitum*, 6 days a week, for 7 weeks. Throughout the experiment, the amount of food consumed and mortality were daily recorded. Fish were fasted for 24h before sampling and were killed by overdose of anesthetic. Total length and total weight were measured and growth parameters were calculated for each treatment. Blood samples were collected from the caudal vein and haematological (haematocrit-HCT, red blood cell-RBC, and white blood cell-WBC) and immunological parameters (complement, anti-protease, myeloperoxidase, alkaline phosphatase, and ceruloplasmin activities), were assessed. Liver samples were collected for the determination of hepatic lesions through histological analysis. Data were analyzed by one-way ANOVA, followed by Tukey's post hoc test at a significance level of $P < 0.05$, using the IMB SPSS Statistics version 26 statistical program. The experimental protocol was approved by the Research Ethics and Ethics Committee of the University of Thessaly.

Results and Discussion

High concentrations of mycotoxins in DON-C and FB-C treatments caused significantly lower food consumption compared to other treatments. Similarly, significantly lower mean weight, mean length, and total biomass increase, were also recorded. In groups FB-A and FB-C, significantly lower mean weight was also estimated, compared to the control group. In DON and FB-A treatments, significantly higher FCR was calculated, while significantly lower SGR was estimated in the DON-B, DON-C, FB-A, and FB-C treatments, compared to the control group. Reduced food intake and limited growth have also been observed in salmon and carp after administration of deoxynivalenol, at similar concentrations (Bernhoft et al., 2018). Also, in the case of gilthead seabream, the dietary administration of FB affected growth parameters (Goncalves et al. 2020). Both mycotoxins affected the examined haematological and immunological parameters. A significantly lower haematocrit was observed in the DON-C group compared to the control group. Differences were observed in the blood cell counts, however, this was not statistically confirmed. Increased myeloperoxidase activity and ceruloplasmin activity were observed in DON-A and DON-C groups and decreased hemoglobin and alkaline phosphatase concentration in the DON-B group. FB addition showed significantly increased myeloperoxidase and ceruloplasmin activities in the FB-B and FB-C

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treatments and a tendency towards decreased alkaline phosphatase activity at all doses tested. None of the mycotoxins tested affected significantly the complement-mediated antibacterial activity or the anti-protease activity of the serum. The myeloperoxidase activity is proportional to the production of peroxide, a potent ROS that may provoke oxidative stress in the fish cells. The ceruloplasmin activity indicates some inflammatory response, while the alkaline phosphatase activity is linked to the anti-inflammatory response. Histopathological examination of the liver from the control group showed normal structure in the liver parenchyma and hepatopancreas. Both DON and FB groups showed mild (DON-A, DON-B, FB-A, FB-B) to extensive (DON-C, FB-C) pathological lesions, such as mild degenerative changes of hepatocytes, with graded hydropic and fatty degeneration, as well as early necrotic changes, with nuclear pyknosis and shrinkage. Mild congestion of the hepatobiliary blood vessels was also observed while the pancreatic cells appeared normal. Liver changes have also been confirmed in the case of deoxynivalenol administration in trout (Gonçalves et al., 2018). In conclusion, the dietary addition of DON and FB showed a dose-dependent negative effect on gilthead seabream growth and health.

Acknowledgment

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Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

EFFECT OF THE ANTITUMORAL DRUG VERTEPORFIN ON THE REPLICATION OF *Spring viraemia of carp virus* (SVCV)

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Introduction

Fish, like other vertebrates, are susceptible to viral infections that cause huge losses to aquaculture. An important disease in the aquaculture sector is caused by the Spring viraemia of carp virus (SVCV) belonging to the *Rhabdoviridae* family, which includes some of the most important viral pathogens in aquaculture (Leong et al., 2008). SVCV is a virus with negative polarity single-stranded RNA genome encoding 5 proteins: 3 -´ Nucleocapsid protein (N)-phosphoprotein (P)-Matrix protein (M)-Glycoprotein (G)-RNA-dependent RNA polymerase (L)-5 ´. Common carp is the main hosts of SVCV and highly susceptible to infection. In European Aquaculture, SVCV causes losses up to 4000 tons annually (Fijan et al., 1999) Fish viruses are harmless to humans and are therefore a risk-free research tool. In addition to its importance in the aquaculture sector, SVCV is easy to grow in carp cells, where a number of interferon-related genes have been identified, thus making this virus a suitable candidate for the study carried out in this work.

Verteporfin (VTPF) is a derivative of benzoporphyrin, extracted from the mugwort plant (*Tanacetum parthenium*), that is used for the treatment of age-related macular degeneration (Wang et al., 2016) as a photosensitizer. Evidence has been found for the action of VTPF on signaling pathways related to the innate immune response. Specifically, Verteporfin has been identified as an inhibitor of YAP activity which is a central effector of the Hippo signaling pathway responsible for regulating organ size and tumorigenesis (Feng et al., 2016). Many immunodeficiency symptoms, including periodic bacterial and viral infections, are associated with deficiency of Hippo signaling, implicating its crucial role in immune modulation (Harvey et al., 2013).

Our goal in this study was to analyze the effect of VTPF in EPC cells infected (SVCV) or immunostimulated (poly I:C), to determine the cell antiviral status under those conditions.

Results

The overall objective of this research was to study the role of Verteporfin, a compound used in the treatment of cancer and degenerative diseases, on the immune response in *Epithelioma papulosum cyprini* (EPC) cells. First, the toxicity of the compound was evaluated using the EPC carp cell line observing that up to 2 µM VTPF is not toxic and a protective effect of VTPF against SVCV was seen at concentrations between 0.5-1 µM when VTPF was present during infection.

In plaque reduction assays, pretreatment of cells with VTPF led to the inhibition of SVCV at 4dpi, but not statistically significant. Addition of the compound at the beginning of the infection (0 h) did not produce inhibition. However, in qPCR experiments inhibition of SVCV N gene RNA synthesis at 24 hpi after VTPF pretreatment, and at 72 hpi under VTPF during the infection (0 h) was observed.

In teleost fish it has been shown that *ifitm* (interferon-stimulated transmembrane protein) genes respond when cells are treated with the synthetic double-stranded RNA poly I:C (a molecule that stimulates the interferon pathway). Our results corroborate this: poly I:C treatment increased 3 to 5-fold the expression of *ifitm1* and *ifitm5*, but also other genes related to the innate immune response such as: *isg15* (interferon 15kDa-stimulated gene) and *irf3* (interferon regulatory factor 3) that were stimulated 2 and 5-fold, respectively. As for the pretreatment of the cells with VTPF, there was an increase in the expression of *ifitm*1, but to a lesser extent *irf3* and *isg15*. It can not be ruled out that other interferon-modulated genes are affected by VTPF treatment, as has been observed in other fish cell lines (Zhang et al. 2021).

In summary, we have shown VTPF antiviral activity against SVCV in both RNA synthesis and cell survival experiments, but not in plaque formation assays. The double treatment of poly I:C + VTPF exerted the highest antiviral activity against SVCV. When cells were treated with VTPF a stimulation of innate immune response occurred.

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Acknowledgements

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PLASTIC LITTER FINGERPRINT ASSESSMENT OF DIFFERENT MULTI-TROPHIC AQUACULTURE SYSTEMS: METHOD DEVELOPMENT AND APPLICATION

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Introduction

There is continued pressure to intensify aquaculture production to fulfil market needs. The expansion of the industry and increased diversity of materials used to build and maintain open, and recirculating aquaculture systems (RAS) have paralleled the development of synthetic polymers over the last decades (Astudillo et al., 2009). Synthetic materials offer greater strength and durability than natural fibres for the construction of ropes, infrastructures, and pipes, whilst often also being less costly and easier to handle. All plastic material within an aquaculture site is maintained and controlled for chemical degradation, biofouling and corrosion, and regularly inspected to ensure strength and stability. Broken and fragmented equipment as well as debris released from intense use however are sources of plastic emission from aquaculture operations at a local and global level, whilst accurate estimations of their contribution remain unknown (Lusher et al., 2017).

The project ASTRAL (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture) focuses on integrated multi-trophic aquaculture (IMTA) farming, aiming at defining, supporting, and promoting this type of sustainable aquaculture production across the Atlantic area. To estimate the environmental sustainability, the fingerprint of different IMTA production systems is needed. ASTRAL is looking at the challenges related to the release of microplastics from aquaculture operations in both open and recirculating systems as well as quantifying the marine derived sources of plastics potentially impacting the IMTA labs. A monitoring plan was designed and applied at selected IMTA labs, using a novel sampling equipment able to preconcentrate large volumes of seawater onto appropriate filtering membranes.

Materials and Methods

Three sites were selected within the present study: A) the coastal open multi-trophic aquaculture facility located in Port-a-Bhuiltin (Scotland) managed by the Scottish Association for Marine Science. Being productive throughout the year, the farm aims at adding shellfish (native oysters, king scallops) to traditional seaweed production; B) the coastal open multi-trophic aquaculture facility located in Bertraghboy Bay (Ireland), managed by the Marine Institute. The farm aims at introducing new species combinations and C) The onshore partially (50 %) recirculating multi-trophic aquaculture facility "Buffeljags Abalone" located near the city of Cape Town, South Africa and managed by Viking Aquaculture. The farm aims at culturing a sub-tropical sea urchin species in temperate seas as well as integrating aquaculture of urchins with Ulva spp. and abalone. The occurrence of plastic fragments in different environmental compartments potentially affected by aquaculture production activities such as seawater and marine sediments was investigated at increasing distances from the open systems aquaculture facilities. The sampling grid was defined in relation to the site's location, and the direction and intensity of the main surface and sea bottom currents present at site. Three sampling sites were selected from each of the open systems. In the partial RAS system, sampling sites were selected in the water inlet, outlet, as well as in some selected areas inside the recirculating system (abalone & Ulva raceways). For the seawater collection a Compact Large Volume Microplastics sampling device was developed. A large volume of seawater is conveyed by a compact stainless steel pump unit to a cascade of two stainless steel filters of 10 and 300 μm mesh size where particles (including micron-sized plastic fragments) are trapped. After each sampling, filters can be easily released from the small portable filter holder unit (fig.1) and accommodated on pre-cleaned glass petri dishes of appropriate size, sealed, kept in cold and dark conditions prior to the sample preparation and analysis steps. The sediments were collected by means of a Van Veen grab device. The first 0 to 5 cm sediments layer was collected for analysis. The extraction and purification method for all samples included a combination of a multistep enzymatic-strong alkali-oriented incubation followed by density-based separation to extract plastic fragments from digestates. Extracted samples were analysed first by μ-FTIR (Fourier Transform Infrared) microscopy and finally by GCMS-pyrolysis technique.

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Figure 1 - Large Volume Microplastics sampling device operating at Bertraghboy Bay sampling site.

Results and Discussion

The preliminary evaluation of the seawater samples shows the occurrence of 27 different polymer types. Among them the most recurring ones were polyethylene (PE), polystyrene (PS), polypropylene (PP), ethyl vinyl acetate (EVA), polycarbonate (PC), polyurethane (PU), (polyvinyl chloride) PVC and polyamide (PA66). The observed fragments were mapped against the IMTA lab inventory of polymers used in their daily operation. The sites targeted as reference in the study showed a similarly representative pool of polymers but with different accumulation levels. The preliminary results point out a complex distribution of polymers which hamper the interpretation of the source of microplastics in the aquatic environment. The ongoing work will contribute towards an improved understanding of the complexity, introduction, and potential emission of synthetic polymers in open and recirculating IMTA systems that will contribute towards the improved sustainability of modern aquaculture systems.

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THE POWER OF SPICES AS DIET SUPPLEMENTS TO BOOST LIPID METABOLISM AND FAT UTILIZATION IN RAINBOW TROUT, *Oncorhynchus mykiss*

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Introduction

Spices, particularly pungent spices (activators of Transient Receptor Potential Vanilloid receptor 1, TRPV1), have long been known to positively influence metabolism, improve energy balance (reduce adiposity) and digestion, especially of fats (Srinivasan, 2005; Westerterp-Plantenga et al., 2006). A multitude of effects, including hypolipidemic and hypocholesterolemic activities, increase of fat oxidation, stimulation of bile acid synthesis and bile secretion, and stimulation of digestive enzyme activities, have been well established in mammals. Therefore, the active principles of many spices are considered excellent nutraceuticals. However, these effects have been little explored in fish. On the other hand, in the context of the need to reduce levels of fish oils in fish diets, alternative oils rich in saturated fatty acids (SFAs) and minimizing tissue deposition of 18:2n-6 have been advocated, especially for salmonids (Fonseca-Madrigal et al., 2005). However, these oils have the potential to affect lipid metabolism and digestibility. Having this in mind, the objective of the present study was to test the potential of spices to improve performance and enhance metabolism of rainbow trout fed high lipid diets with a standard oil composition or, alternatively, enriched in SFAs.

Materials and methods

A 100 days trial was performed with 4 groups of rainbow trout (start weight: 61.9±8.9g) in 4 replicate 500L tanks. Fish were fed isoproteic and isolipidic diets (42.5% crude protein and 27.2% crude fat) with either a standard (STD) composition of added oils (7.38% fish, 7.38% soybean, 7.38% rapeseed and 2.46% linseed oils) or a more saturated (SAT) composition (4.92% fish, 4.92% soybean, 4.92% rapeseed and 9.84% palm oils). Two control groups were fed one of the basal diets (CTR-STD or CTR-SAT) and two treatment groups were fed the same diets supplemented with an encapsulated mixture of spices oleoresins at 0.075% (SPI-STD or SPI-SAT). At the end of the growth trial, a lipid digestibility study was performed, and 8 animals per treatment were sacrificed and samples were collected for fillet composition, liver and intestine histological and gene expression (by qPCR) analysis, and bile and liver bile acids (by HPLC) analysis. Results were analyzed by two-way ANOVA considering the effects of "Diet", "Treatment" and their interaction. Values shown in graphs are means ± standard error of the mean (SEM).

Results and discussion

A significant improvement of body weight (BW) was observed at day 72 when diet was supplemented with SPI ($P<0.05$), but at the end of the trial only a trend was noticeable ($P=0.08$ for the comparison CTR-SAT *vs.* SPI-SAT, +5.5% BW in the later treatment). A significant interaction was found for feed conversion ratio (FCR), which was only significantly improved with the addition of SPI in SAT diets (t-test $P=0.01$, -12.7% in SPI-SAT *vs.* CTR-SAT). No significant effects were found in perivisceral fat level or hepatosomatic index but scores of lipid accumulation in hepatocytes, assessed histologically, were significantly reduced by the SPI additive in both diets. No changes were observed in histological scores of lipid accumulation in enterocytes but the average size of lipid droplets was noticeably reduced. Fillet proximate composition was not significantly affected, albeit % lipids was numerically reduced by SPI supplementation.

Lipid digestibility was not significantly affected, although a slight numerical increase (+1.4%) was observed in the SPI-SAT compared to the CTR-SAT treatment.

Analysis of gene expression in liver indicated several genes that were significantly affected by SPI supplementation (i.e., "treatment"), including genes involved in lipogenesis, β-oxidation, and their regulation (PPARα), as well as genes involved in bile acid synthesis and transport. Similar results were observed in intestine. However, results of bile acid concentration in liver and bile were highly variable (no significant changes).

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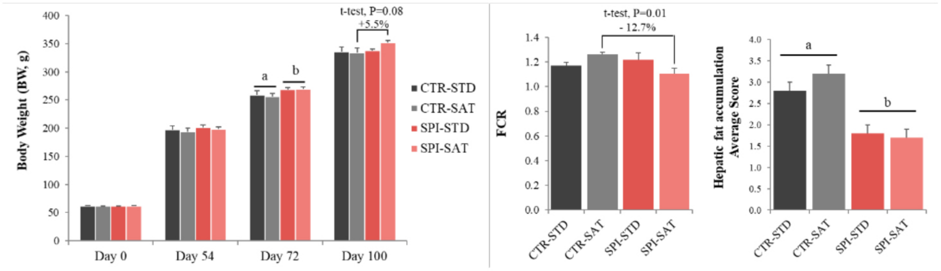

Fig. 1. Fish BW during the growth trial (a), FCR (b) and hepatic accumulation scores (1-5) (c) at the end of the trial.

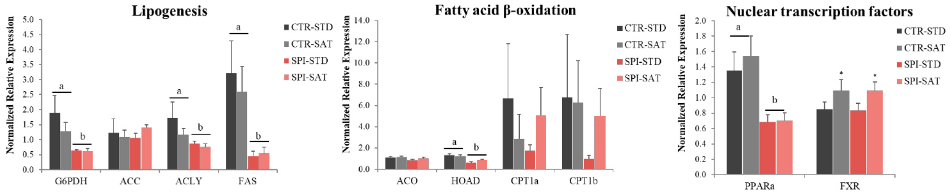

Fig. 2. Hepatic expression of lipid metabolism genes, assessed by qPCR. Different letters denote P<0.05 for "Treatment" and asterisks for "Diet" factors.

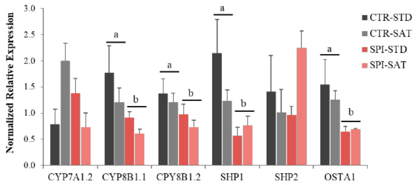

Fig. 3. Hepatic expression of bile acid synthesis genes, assessed by qPCR. Different letters denote P<0.05 for "Treatment" and asterisks for "Diet" factors.

In conclusion, present results confirm the hypotriglyceridemic effect of spice oleoresins in trout, denoted by a lower lipid accumulation in hepatocytes. Such effect has been well demonstrated in mammals for pungent spices, which prevent the accumulation of fat in liver by enhancing triglyceride transport. As in mammals, a strong activation of fatty acid metabolism was observed. Part of the hypolipidemic and hypocholesterolemic effects of spices is attributed to an enhanced cholesterol turnover to bile acids through activation of bile acid synthesis enzymes. This has also been observed in the present study. Metabolic effects were independent of basal diet formulation. However, noticeable improvements in fish performance (growth and especially FCR) were only seen in the more challenging CTR-SAT diet containing higher levels of SFAs.

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ALTERNATIVE ASSESSMENT OF FISH AND SHRIMP GUT HOMEOSTASIS AND PERFORMANCE: AN EX-PLANT MODEL TO WEIGH THE EFFECTS OF FUNCTIONAL INGREDIENTS

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Introduction

Gastrointestinal tract homeostasis is key for animal health, and in aquaculture, fish and shrimps are frequently challenged with conditions that disturb gut functional stability (e.g. water quality, stressors, antinutritional factors). Assessing gut condition upon an insult is a central task to develop and test novel ingredients with damage mitigation properties. *In vivo* trials are the classical approaches; however, the *in vivo* simulation of gut homeostatic disruption might present ethical barriers, and the time frame required for these types of assessments is long. There is here an opportunity to introduce alternative approaches, thus, the aim of this work was to standardize and implement a fish and shrimp gut *ex vivo* based model to evaluate intestinal homeostasis regulation by novel ingredients, targeting in particular the intestinal immune and antioxidant response and epithelium integrity for permeability regulation.

Material and Methods

An ex-plant culture of intestine was performed for both fish and shrimp to assess acute disruption and potential mitigation by functional ingredients. Juvenile gilthead seabream (*Sparus aurata*) and whiteleg shrimp (*Penaeus vannamei*) were chosen as models and after standardization steps (e.g., medium choice, incubation time), a panel of selected genes was assessed to enrich the model with a cost- and time-effective quantification of the gut health status. The panel included genes related to immune (e.g. *cox2*, *IgM*) and antioxidant response (e,g, *cat*, *gpx*), but also related to epithelium integrity (e.g. *tjp2*, *cldn12*). To evoke a response representative of dysregulation, different insults were included as proxies. Here we simulated a bacterial infection (lipopolysaccharide), an oxidative damage (hydrogen peroxide), exposure to a toxic compound (dextran sulfate sodium) as well as to an antinutritional compound (soy saponin). The expression of the panel genes was assessed and response to insults was compared with the response to several functional ingredients (e.g. algae), including commercially available ones (e.g. medium chain fatty acids, butyrate).

Results and Discussion

The intestinal ex-plant culture represents an alternative tool to screen the potential of ingredients as a gut homeostatic dysregulation mitigators or protectors. In this study we observed a differential response to the different insults that during the acute phase could be representative of the main effects exerted on the gut mucosa. The effects of the lipopolysaccharide in the anterior intestine were evidenced by a strong upregulation of *cox2* and *IgM* among others, indicating activation of the immune response. On the other hand, soy saponins response was associated with an upregulation of the gene coding for pro-inflammatory enzyme (*cox2*), the glutathione peroxidase (*gpx*) and catalase (*cat*), indicating an immune and antioxidant response that was expectable through the action of this antinutritional compound known to promote intestinal inflammation in fish. Soy saponins also triggered an increase in the expression of genes related to the tight junctions' complex (e.g. occludin) in both intestinal sections, as a compensation for a possible disruption. These and other results allowed to categorize and separate the tissue's response to the different insults and served as standards for grading the response to functional ingredients. Here we identified algae immunostimulatory potential, and medium chain fatty acids buffering of epithelium integrity through the regulation of tight junction's related genes. Regarding shrimp intestinal explant, an evident immune response was recorded after incubation with lipopolysaccharide, showing its potential for further nutritional studies. These promising results gave ground to a wider exploration of this alternative tool for functional ingredients assessment. The gene panel is under expansion to unravel the underpinnings of the intestinal homeostasis regulation. Further, since one of the main assets of the ex-plant approach is the ability to reveal a fast response to the stimuli, further studies are in place to evaluate synergies and antagonisms between functional ingredients and its dosages. This will allow a fine tuning of the functional ingredients to include in a formula considering the target feature of the diet that will be tailored (e.g. stress mitigation, immunostimulation).

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ANTIGEN-DISPLAYING *Bacillus subtilis* SPORES PROTECT ZEBRAFISH LARVAE AND EUROPEAN SEABASS JUVENILES UPON BACTERIAL INFECTION

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Introduction

Aquaculture production continues to grow steadily within the food production sector, but one major drawback to its sustainable growth and profit is the emergence of infectious diseases. There is a current need for finding alternative vaccination methods since most commercially available vaccines are injectable, which require laborious work and can cause stress-related mortalities in the fish. Immersion and oral vaccination seem to be the most suitable options for antigen mass delivery. In particular, oral vaccination through the incorporation of the vaccine in the fish feed would reduce fish stress, vaccination costs and is suitable for not only juvenile and adult fish but also larvae stages (1). *Bacillus* spores are the ideal candidates for oral antigen delivery, since its resistant structure allows not only the survival to harsh conditions of the gastrointestinal tract but the incorporation into the fish feed (2). Surface display of antigens has already been applied with promising results for developing oral vaccines for fish, in particular by using *Bacillus subtilis* spores to display immunogenic proteins from virus and gram-positive bacteria (3,4), but to date, this technology has not been applied to fight fish diseases caused by gram-negative bacteria.

In this work, we used *Bacillus subtilis* 168 spores as vehicles for the delivery of four immunogenic proteins, and its protective potential was evaluated in increasing the survival of zebrafish larvae challenged with *Vibrio anguillarum*, *V. parahaemolyticus, Aeromonas hydrophila*, *Edwardsiela tarda* or *Photobacterium damselae* subsp. *piscicida*. The potential of one vaccine candidate for vibriosis was also evaluated in European seabass.

Materials and Methods

Animal experiments and handling were approved by the Animal Welfare Committee of the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), performed by trained scientists (with FELASA category C), and carried out in a registered installation (N16091.UDER), in compliance with the European directive 2010/63/EU for the care and use of laboratory animals.

Four immunogenic proteins were tested in this work: OmpK, an outer membrane protein shared by several *Vibrio* spp., Php_0080, a lipoprotein found in the membrane of *Photobacterium damselae* subsp. *piscicida*, FlgD, a flagellar protein from *Edwardsiela tarda* and GAPDH, an outer membrane protein used for developing polyvalent vaccines. The genes encoding these proteins were amplified and cloned into p1CSV-CotY-N and p1CSV-CotY-C plasmid vectors (5), resulting in C-or-N-terminal fusions to the crust protein CotY. The integration of the translational fusions in the *B. subtilis* 168 chromosome resulted in the congenic derivatives strains: CRS220 (CotY-H6-OmpK), CRS221 (H6-OmpK-CotY), CRS226 (H6-Pdp_0080-CotY), CRS227 (H6-GAPDH-CotY), CRS228 (CotY-H6-GAPDH), CRS229 (H6-FlgD-CotY), CRS230 (CotY-H6-Pdp_0080) and CRS231 (CotY-H6-FlgD). Spores of the parental strain *B. subtilis* 168 and its congenic derivatives were produced and purified following standard procedures, and the display of each target immunogenic protein was assessed by western blot. The vaccination potential of the antigen-displaying spores was evaluated in zebrafish larvae at 28ºC, distributed in 6-well plates (10 larvae per well) containing egg water. At 6 days post-fertilization (dpf), larvae were exposed for 2h with 10^8 CFU mL^{-1} of each recombinant spores suspension. At 9 dpf larvae were challenged by immersion with 3×10^8 CFUs mL^{-1} of *V. anguillarum*, 1×10^8 CFUs mL^{-1} of *V. parahaemolyticus*, 8.5×10^7 CFUs mL^{-1} of Ph

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damselae subsp. *piscicida*, 1×10^8 CFUs mL^{-1} of *E. tarda* or 1×10^{10} CFUs mL^{-1} of *A. hydrophila*. Cumulative mortalities were registered between 16 and 24h and each experiment was carried out 3 times. The protective potential of OmpK-displaying spores was also evaluated in European seabass. Triplicate groups of juveniles were fed diets that incorporated 1×10^9 spores Kg^{-1} feed of either CRS220 (OmpK-diet) or from the parental *B. subtilis* 168 strain (CTR) for 30 days. Sixty fish fed each diet were challenged by intraperitoneal injection with $1x10^6$ CFU/fish of *V. anguillarum*. Fish injected with PBS were included as a control, and fish survival was followed for 7 days. Survival curves were plotted using the Kaplan-Meier method and pairwise comparisons between treatments were performed with a nonparametric log-rank test, at 0.05 significance level, in GraphPad Prism version 9.

Results

Zebrafish survival upon challenge with *V. anguillarum* and *V. parahaemolyticus* increased in magnitudes of 50 to 90% respectively, when previously vaccinated with CRS220 (CotY-OmpK) spores. Survival significantly increased when larvae were treated with CRS230 and CRS231 and challenged with *Ph* subsp. *piscicida* and *E. tarda*, respectively. Larvae treated with spores from CRS228 (CotY-GAPDH) significantly increased survival upon challenge with *A. hydrophila*, *E. tarda* and *Ph* subsp. *piscicida*, showing the polyvalent potential of GAPDH-carrying spores. Further, European seabass fed diets containing OmpK-carrying spores increased survival by 30% when challenged with *V. anguillarum*.

Conclusions

These results confirm the potential of antigen-displaying spores as immersion and oral vaccines in zebrafish larvae, a model organism, and European seabass, an economically important aquaculture species.

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EFFECTS OF THE DIFFERENT STIMULUS PERIODS FROM FIRST FEEDING ON VEGETALE-BASED DIET CHALLENGE IN ATLANTIC SALMON (*Salmo Salar. L*) FINGERLINGS

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Introduction

The main source of omega 3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), such as eicosapentaenoate (EPA) and docosahexaenoate (DHA) in aquafeeds is fish oil, which is currently a limited and finite source. Nutritional programming that induces long-term consequences on animal metabolism and physiology, such as biosynthesis of LC-PUFA (Lucas, 1998), could be adopted for sustainability. Previous studies have confirmed that the pathway for EPA-DHA synthesis was upregulated in Atlantic salmon populations first-fed vegetable-based feeds during a 3-week stimulus (Vera *et al*., 2017), combined with improved growth rate and food conversion efficiency (Clarkson *et al*., 2017). Thus, the present research evaluated if nutritional programming changes triggered by an early nutritional intervention are still prevalent after 16 weeks and whether a new challenge will enhance the tissue net production of EPA and DHA as a booster.

Material and Methods

In the present trial two types of diets were applied to salmon from first feeding (initial weight 0.15 g): a marine diet comprising mainly fish meal and fish oil (EPA+DHA 26% total fatty acids; TFA), or a vegetable-based diet containing plant proteins and vegetable oil (EPA+DHA 5.9% TFA). As shown in figure 1, at the first feeding "stimulus phase", three experimental groups (300 fish/tank, n=3) were fed either with a vegetable-based diet for one or two weeks (V1 and V2, respectively or the marine diet (M) individually to provide stimulus as a program. Afterwards, all experimental groups were fed M diet until the end of 16th week (marine phase) before all treatments were transferred back to the vegetable-based diet for 6 weeks (challenge phase). Fish were sampled and tissues collected for lipid analysis (liver, brain, eye, gills, intestine and muscle), gene expression (liver), as well as histology (liver and intestine) at the end of the marine and challenge phases.

Results

The challenge diets reduced the n-3 LC-PUFA contents, as expected, in the total lipids of all tissues ($p<0.05$). On the other hand, the difference of the n-3 LC-PUFA amount in the total lipid between treatments in all of the tissues were insignificant ($p>0.05$) for both stages. Similarly, the n-3 levels in tissue polar lipid were not clearly influenced by treatments ($p>0.05$) but a significant decrease in n-3 levels after challenge was observed ($p<0.05$). In liver, V1 showed a significant up-regulated expression for fatty acid desaturase and elongation of very long chain fatty acids than V2 only at the end of the challenge ($p<0.05$). On the other hand, the transcription factors sterol regulatory-element binding proteins 1 and 2, liver X receptor as well as peroxisome proliferator activated receptor alpha and gamma, were significantly down-regulated in V2 ($p<0.05$). The regulation of lipid metabolism related genes, fatty acid synthase, acyl-CoA oxidase and carnitine palmitoyltransferase I shared the similar pattern as M > V1> V2 at the end of the challenge phase ($p<0.05$). The percentage of the hepatic vacuolization in group V2 was significantly higher than group M for both stages ($p<0.05$). In the anterior intestine, V1 showed the slightly lower amount of goblet cells after marine phase ($p>0.05$) which became significant after challenge ($p<0.05$).

Discussion

The decrease of EPA, DHA and n-3 LC-PUFA in tissues after challenge can be explained by the diet change. Comparing the individual fatty acid content between the total lipid and polar lipid of tissues indicates the deposition preferences of LC-PUFA from the intake or endogenous production. Also, the less impacted fatty acids after challenge suggest their functional priority. In the present study, the relatively stable content of DHA in liver may be the result of high involvement for structural significance such as membrane formation (Sargent *et al*., 2002). In addition, EPA and DHA could be endogenously biosynthesized from their precursor 18:3n-3 (Tocher, 2003), as confirmed by the gene expression results. Compared with vegetable diet challenge phase, the different early stimulus periods had rare impact on the fatty acid composition. However, further molecular analysis indicated that several LC-PUFA biosynthesis regulators showed to be up-regulated in V1, while the down-regulation found in most transcription factors in V2 group suggested a negative influence of the two-weeks stimulus.

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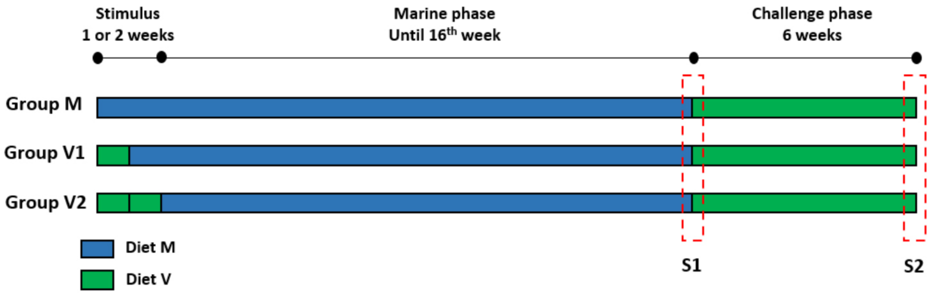

Figure 1. Experimental design: S1 (Sample point 1), end of marine phase. S2 (Sample point 2), end of challenge phase.

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EFFECT OF MEDICINAL PLANTS, YEAST AND *Bacillus licheniformis* ON THE GROWTH, SURVIVAL, IMMUNE RESPONSE AND DIGESTION OF SHRIMP (*Penaeus vannamei*) CHALLENGED WITH *Vibrio parahaemolyticus*

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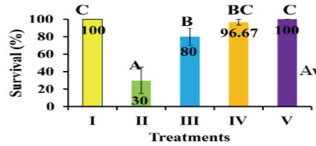
In shrimp farming viral diseases produce important economic losses. The use of prophylactic methods as natural additives wthout inmmune resistance and environmental problems were proben. Medicinal plants powder, yeast and *B. licheniformis* were used in the survival in the digestive and immune systems of *P. vannamei* challenged against *V. parahaemolyticus*.

Four bioassays were carried.Postlarval white shrimp stage were used to analyze the digestive and immune-related genes expression by RT-qPCR.

The combination of MP in the food and *B. licheniformis* in the water (3 x 106 CFU / L) significantly improved the survival of *P. vannamei* challenged with *V. parahaemolyticus*. *B. licheniformis* inoculated in the water, did not alter the expression of the trypsin digestive gene.

B. licheniformis in the water decreased the expression of the SOD gene (related to the immune system), which plays an important role as an antioxidant, decreasing the concentration of superoxide anion, the product of the phagocytosis process. *B. licheniformis* in the water did not alter the expression of the genes of the immune system penaeidine4 and lysozyme. The mixture of MP added in the feed and *B. licheniformis* in the water prevent the AHPND in *P. vannamei* cultivated in the laboratory.

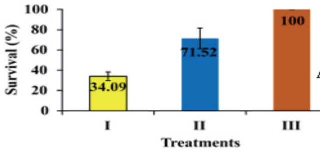
Bioassay 1. Medicianl Plant (MP) powder added to feed every 2 d and two concentrations of *B. licheniformis* in the water after 7 d were evaluated.



CL$_{50}$: 180,000 UFC/mL.
Average media weigth = 0.56 g

1) Control (-); II) Control (+); III) MP 4 g/kg c/2 d + Vibrio CL$_{50}$; IV) BL 1 x 10^{6} UFC/L c/7 d + Vibrio CL$_{50}$; V) MP 4 g/kg c/2 d + BL 1 x 10^{6} UFC/L c/7 d + Vibrio CL$_{50}$.

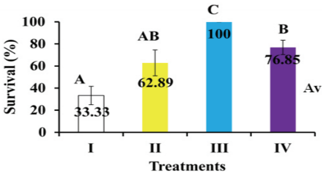
Bioassay 2. Best results with MP and *C. parapsilopsis* were added to feed after 2 d and *B. licheniformis* in water after 3 d.



CL$_{50}$: 105,000 UFC/mL.
Average final weigth = 0.344 g

I) Control (+); II) MP 4 g kg^{-1} c/2 d + BL *2 x 10^{6} UFC L^{-1}* 3 d^{-1} + *Vibrio* CL$_{50}$; III) MP/2 d^{-1} + BL *3 x 10^{6} UFC L^{-1}* 3 d^{-1} + *Vibrio* CL$_{50}$.

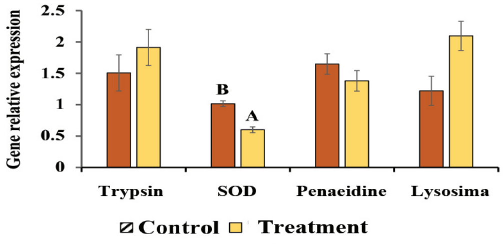
Bioassay 3. The yeast added to feed each 2 d and *B. licheniformis* in water after 3 d were analizaed.



CL$_{50}$: 63,000 UFC/mL.
Average final weigth = 0.173 g

Control (+) ; II) Yeast 2 g kg^{-1} 2 d^{-1} + *Vibrio* CL$_{50}$; III) BL 3 x 10^{6} UFC L^{-1} 3 d^{-1} + *Vibrio* CL$_{50}$; IV) DKT 3 g kg^{-1} 2 d^{-1} + *Vibrio* CL$_{50}$.

Bioassay 4. The yeast in two different concentrations and *B. licheniformis* in the water. All four bioassays were challenged against *V. parahaemolyticus*.



BL 3 x 10^{6} UFC L^{-1} 3 d^{-1}

EVALUATING ENZYMATIC PRODUCT APPLICATION FOR THE BREAKDOWN OF HYDROGEN PEROXIDE FOLLOWING ROUTINE TREATMENTS IN AQUACULTURE SYSTEMS

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Introduction

Water treatments and therapeutants applied in recirculating aquaculture systems (RAS) are complicated by potential impacts on critical nitrifying bacterial populations in biofilters (Noble & Summerfelt, 1996), specifically reduced oxidation of total ammonia nitrogen (TAN) and nitrite-nitrogen. Hydrogen peroxide (H_2O_2), while considered environmentally benign due to non-toxic end-products (Block, 1991), is known to disrupt biofiltration when applied at therapeutic concentrations in RAS (Fredericks, 2015). Previous research has assessed low-dose H_2O_2 applications in RAS to avoid biofilter impacts (Møller et al., 2010; Pedersen et al., 2012); however, the ability for RAS producers to use therapeutic or disinfection-level H_2O_2 concentrations in culture tank static bath treatments, followed by decomposition of H_2O_2 prior to resumption of recirculating water flow through biofilters, would be advantageous. We therefore sought to assess H_2O_2 decomposition using a novel enzymatic product following static bath applications of H_2O_2 at both therapeutic- and disinfection-level concentrations.

Materials and methods

Two single-day experiments were conducted to assess the effectiveness of a new catalase enzyme product, BioRas® Balance, in the decomposition of H_2O_2 into H_2O and O_2, applied as a static bath at either therapeutic- or disinfection-level concentrations. The first trial (Trial 1) employed simulated static bath treatments for bacterial gill disease in replicated culture tanks, with live fish present, followed by application of BioRas® Balance in a range of concentrations to assess dose-response decomposition of H_2O_2 over time. Trial 2 was a similar dose-response experiment, but employed disinfection-level H_2O_2 concentration with no live fish present. *Trial 1*. Diploid Atlantic salmon *Salmo salar* were received as fertilized eggs and hatched onsite using a temperature-controlled incubation system, and then cultured to market-size in freshwater RAS. To begin the study, 216 fish (5 kg mean weight) were stocked randomly into 12 replicated partial recirculating aquaculture systems (PRAS). Each PRAS consisted of a 5.0 m^3 dual-drain tank, a gas conditioning column with counter current forced air ventilation, and a low head oxygenator (LHO) with a sump. A 0.4 horsepower pump, located in the sidewall box of each tank, continuously recirculated 379 L/min of water. Biomass densities at the start of the experiment were approximately 20 kg/m^3. PRAS were operated at a 90% recirculation rate (on a flow basis), and under these conditions typical water quality parameter values for both studies were as follows: temperature (14 °C), pH (7.8), alkalinity (277 mg/L), CO_2 (4.5 mg/L), TAN (0.2 mg/L), and total suspended solids (1.0 mg/L). Following acclimation, 60-minute static bath tank treatments were carried out, simulating therapeutic treatment for bacterial gill disease (100 mg/L H_2O_2 applied for approximately 60 minutes' duration). BioRas® Balance was then added to the culture tanks to create concentrations of 1.5, 2.5, 5.0, and 10.0 mg/L (target concentrations were selected based on previous unpublished research); each tank was randomly assigned one of these BioRas® Balance concentrations, for a total of three replicates per treatment concentration. Following the addition of the catalase enzyme, water samples were collected and H_2O_2 concentrations analyzed at 10 minute intervals, until H_2O_2 concentration within a given tank was less than 0.5 mg/L. *Trial 2*. The second trial was carried out to simulate a typical disinfection event using a higher concentration of H_2O_2 (i.e., 250 mg/L initial target concentration). Dosing of H_2O_2 and BioRas® Balance, as well as H_2O_2 concentrations assessments, were carried out in an identical manner to the procedures used in Trial 1. The only major differences with Trial 2 were that i) water continued to recirculate within the PRAS (as opposed to a culture tank static bath), and ii) no fish were present in the culture tank. Initial and final H_2O_2 concentrations during the pre-catalase, disinfection phase were 261.0 ± 15.3 mg/L and 244.9 ± 14.1 mg/L, respectively.

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Results

In both trials, enzymatic decomposition of H_2O_2 followed a dose-response relationship, catalyzing a first order decay reaction. First-order decay constants and kinetic reaction formulae for each BioRas® Balance application concentration were calculated using least-squares regression of the log-transformed H_2O_2 concentration data; reaction rate constants calculated for Trial 1 were in close agreement to those calculated in Trial 2, indicating that the reaction rate constant may be independent of the initial concentration of H_2O_2. Overall, the results of these experiments are encouraging for RAS farmers who may require utilizing H_2O_2 at concentrations that would normally compromise biofilter nitrification processes. Under the conditions of our studies, BioRas® Balance was effective at quickly reducing H_2O_2 to safe concentrations, in either post-static bath therapeutic application or post-system disinfection scenarios.

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OPTIMISING CAPTIVE BRED BALLAN WRASSE (*Labrus bergylta*) BROODSTOCK PERFORMANCE

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Introduction

Cleaner fish, such as ballan wrasse (*Labrus bergylta*) have proven to be an effective environmentally friendly alternative to chemicals, to control sea lice (*Lepeophtheirus salmonis* and *Caligus spp*) infestations in commercial Atlantic salmon (*Salmo salar*) aquaculture farming (Roth, 2000; Mordue & Pike, 2002; Rae, 2002). Otter Ferry Seafish Ltd. (OFS) started breeding ballan wrasse in 2009. The stocking densities over the past 13 years have not been consistent, with variations in sex ratio and total number of broodfish used per tank. Female broodfish also range in weight from 500g – 3,000g. A collaboration between the Institute of Aquaculture (IoA) at University of Stirling, and Otter Ferry Seafish Ltd., is experimenting in optimising captive bred ballan wrasse broodstock performance.

The aims of this experiment are to prove what stocking density is the most productive, and whether female weight influences productivity. Due to competitive behaviour amongst both males and females, it is hypothesised that a lower stocking density is more productive than at higher stocking densities, and that heavier females produce more eggs, and therefore are more productive, than smaller ones.

Methods

Over a 12-week period, broodfish were stocked into varying size cohorts and allowed to naturally spawn. The ratios were female 20:5 male, female 25:6 male, female: 32:8 male. Each ratio was triplicated.

Over another 12-week period, broodfish were separated into three different size cohorts and allowed to naturally spawn. The treatment sizes were: 520g – 750g, 750g – 1,100g, 1,100g – 2,400g, and mixed sizes. Males were of random sizes. Each treatment was triplicated.

	Female 20:5 Male	Female 25:6 Male	Female: 32:8 Male
Egg Factor (average)	4.5	4.8	4.4
Fertilisation (percent)	58.5	43.6	52.6

Figure 1: the egg productivity (factor) and fertilisation (%) for stocking densities: female 20:5 male, female 25:6 male, and female 32:8 male.

Egg Productivity	Mixed	520-750g	750-1100g	1100-2400g
Biomass (g)	56619	39650	55696	82322
Av	3.7	1.7	3.6	4.2
Sd	3.7	3.2	4.5	4.7
Fert 24h (%)	Mixed	520-750g	750-1100g	1100-2400g
Av	48.8	65.5	57.1	51.8
Sd	40.5	33.3	34.3	35.2
	Mixed	520-750g	750-1100g	1100-2400g
Total larvae	884000	563000	1064000	1562500

Figure 2: egg productivity (factor), fertilisation (%), and total number of larvae hatched for the four female size cohorts: mixed, 520-750g, 750-1100g, and 1100-2400g.

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Results

The number of eggs produced are expressed as a factor where 1 = 12,000 eggs. The results for the stocking density trial are depicted in figure 1 below.

Although the medium stocking density produced the greatest number of eggs (4.8), because its fertilisation was so low (43.6%), the lowest stocking density was the most productive has it had a far higher fertilisation rate (58.5%).

The results of the female size trial are summarised int Figure 2 below.

It was concluded that the largest female broodfish (total biomass of 82,322g) are the most productive as they produce the greatest volume of eggs (egg factor 4.2) and consequently the biggest quantity of larvae (156,2500).

CONSUMERS' VALUES-ATTITUDES-BEHAVIOUR (VAB) TOWARDS CONSUMING SEAWEED FOOD PRODUCTS: THE EFFECT OF PERCEIVED NATURALNESS, UNIQUENESS, AND BEHAVIOURAL CONTROL

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Introduction

Seaweed is a food resource with a neutral environmental impact, as its cultivation helps capture CO_2 and helps the global production of O_2. Therefore, seaweed's cultivation helps counter global warming and is good for capturing and storing CO_2 in the long term; thus, it can be considered a pro-environmental source of food. Seaweed is also of nutritional interest, as most species contain protein, are low in lipids, and low in calories. Seaweed is also known for being rich in iodine, antioxidants, vitamins, and minerals (Mabeau & Fleurence, 1993; Roohinejad et al., 2017).

The consumption of seaweed has been cultivated and consumed in Asia for centuries. In other Western countries, seaweed cultivation is receiving increased interest for both its nutritional and environmental qualities. However, few studies have focused on the factors influencing consumers' behavioural tendencies towards seaweed food products. This study aims to fill the gap by investigating the antecedent of consumers' attitudes and consumption of seaweed food products in Norway. An extended version of the value-attitude-behaviour (VAB) theory is used as a conceptual framework to study seaweed consumption from a social dilemma perspective: hedonistic values and perceived uniqueness versus biospheric values and perceived naturalness.

Materials and methods:

This study uses a representative sample of Norwegian consumers (N = 426). Structural equation modelling was used to test the hypothesis.

Results:

Our results showed that attitude significantly affected the consumption of seaweed food products and that perceived behavioural control positively moderated the attitude–consumption relationship. Perceived naturalness and perceived uniqueness are associated with attitudes towards seaweed. Biospheric values directly influence attitude, while hedonistic values are indirectly related to attitude through uniqueness.

Beyond the theoretical contributions, this study will help the seaweed industry develop its marketing strategy by promoting its naturalness, healthiness, and pro-environmental attributes. Marketers should also make efforts to encourage consumers to associate seaweed with pleasure. Finally, since seaweed food products are perceived as unique, seaweed food products can be positioned as luxury products. By extension, the findings can be used to promote seaweed to policymakers and investors. The European seaweed cultivators remains small, new and requires more private investment and public support to develop. Therefore, promoting seaweed uniqueness and naturalness and emphasizing positive biospheric consequences are ways to influence policymakers and investors positively.

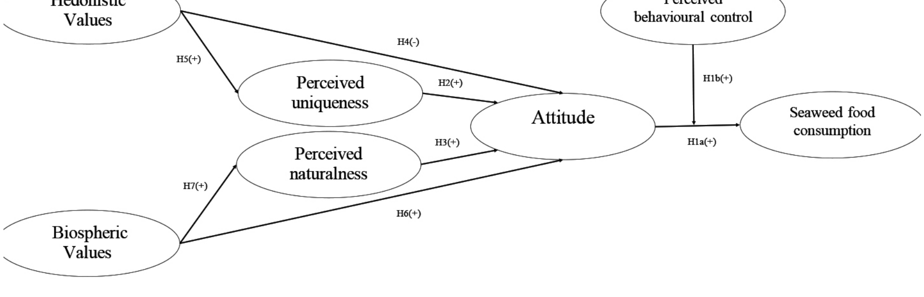

Figure 1. *The proposed structural model with hypothesises*

References:

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A DECISION SUPPORT SYSTEM FOR AQUACULTURE REGULATION IN NOVA SCOTIA

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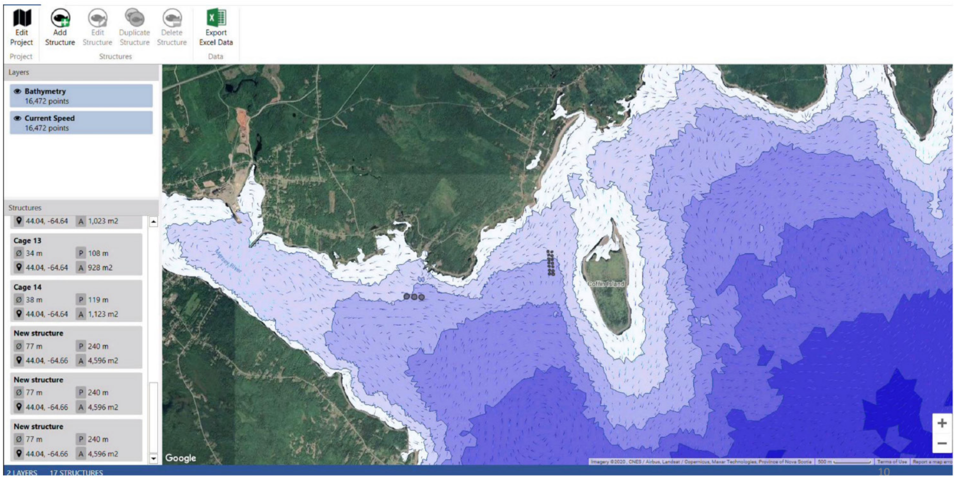
The development of ecosystem models in coastal marine waters containing fish farms has matured to the extent that predictive modelling can be applied to dissolved and particulate waste dispersal, benthic deposition and diagenesis, and the spread of pathogens. These model results are critical to both siting and ongoing monitoring of fish farms. Nonetheless, systematic application of these models to fish farm management is less common, i.e. modelling capability and results are neither widely available or user-friendly. Drawing on decades of model development, we have developed a Windows-based application known as FINS (Farming in Natural Systems) to integrate multiple carrying capacity criteria into aquaculture decision support (Figure 1). FINS is effectively a GIS framework allowing a marine spatial planning approach to management in terms of cage density and location. The following models are included for carrying capacity: (a) Particulate deposition and mapped footprints, (b) sediment diagenesis including sulfides, (c) nutrient plumes, (d) disease risk, and (e) chlorophyll depletion (bivalve culture). Due to the overall forcing of marine environments with circulation via wind and tides, numerical circulation models are the foundation of ecosystem models. We utilize FVCOM as the physical platform to enable diffusion-advection of particles and solutes, and include its particle tracking module to create numerical experiments with respect to non-conservative pathogens such as sea lice. Although all of the component models in FINS are fully developed and groundtruthed, several steps remain including the establishment of carrying capacity thresholds, and integration into the software platform. Among the models, disease risk requires further consideration of epidemiological criteria associated with location of multiple farm sites. A targeted project of the Atlantic Fisheries Fund and the province of Nova Scotia will lead to completion of the FINS framework and extension to multiple sites. Compilation of GIS, bathymetry, river flow, wind fields, and other data is proceeding as input to application to multiple sites in Nova Scotia based on aquaculture development priorities. A calibrated FVCOM model is required for each new location. Although each case study is site-specific, FINS can be developed for any site worldwide.



Bathymetry
16,472 points

Current Speed
16,472 points

44.04, -64.64 A 1,023 m2

Cage 13
Ø 34 m P 108 m
44.04, -64.64 A 928 m2

Cage 14
Ø 38 m P 119 m
44.04, -64.64 A 1,123 m2

New structure
Ø 77 m P 240 m
44.04, -64.66 A 4,596 m2

New structure
Ø 77 m P 240 m
44.04, -64.66 A 4,596 m2

New structure
Ø 77 m P 240 m
44.04, -64.66 A 4,596 m2

2 LAYERS 17 STRUCTURES

Figure 1. Initial view of FINS software showing fish farm location to the left of the island. Shaded bathymetry and current velocity vectors are shown. For scale the island is approximately 1km in length. The left panel shows each cage location and size, and cages may be dragged to any location. The component biogeochemical and disease models are not shown into this screenshot.

TOWARD BENTHIC HEALTH IN SHELLFISH PRODUCTIVE AREAS: EVALUATION OF THE MEDITERRANEAN SEA CUCUMBERS *Holothuria tubulosa* AND *Holothuria polii* AS EXTRACTIVE SPECIES IN CO-CULTURE WITH THE MUSSEL *Mytilus galloprovincialis*

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Introduction

Polyculture of mussel and sea cucumber species is highly attractive since both these organisms have important economical and environmental value. Mussel farming provides more than 1/3 of EU aquaculture products, being one of the most spatial-extended industrial production of mollusk in Europe (Tamburini et al., 2020). On the other hand, the sea cucumbers are considered luxury seafood, especially in Asian countries (Purcell et al., 2016). Moreover, mussel and sea cucumber co-culture potentially doesn't need external food input resulting an environmentally friendly alternative as a high-quality protein source. Mussels, in fact, are filter-feeders able to feed on natural primary production from the water column, without other food supplies (Tamburini et al., 2020). They represent a fundamental organic material connection between the bottom and the water column, by settling organic matter on the seabed. This organic biodeposit could be an attractive food source for deposit feeder sea cucumbers, which are able to ingest sediment and organic material, playing an important role in cycling and energy flow in benthic ecosystems (Purcell et al., 2016). Although the suitability of mussel and sea cucumber polyculture have been investigated in Indo-Pacific (Slater et al., 2007; Zamora et al., 2016), there is no investigation for Mediterranean species. Hence, for the first time in the present study, an Integrated Multi-trophic Aquaculture among Mediterranean species of sea cucumbers (*Holothuria tubulosa* and *Holothuria polii*) and mussels (Mytilus galloprovincialis) was investigated.

Materials and Methods

During the Spring of 2020, a IMTA experiment was carried out at Ittimar Soc. Coop. mussel farm, a *Mytilus galloprovincialis* long-line submerged productive system (South of Adriatic Sea, Italy). Specimens of *H. tubulosa* and *H. polii* were collected along Apulian coast and sorted in two size classes (small size: 40-100 g; large size: 100-200 g). For each species, representative specimens of both size classes were placed in 20 seabed cages beneath the long-line mussel farm at a density of 670 g/m^2. The suitability of *H. tubulosa* and *H. polii* to use *M. galloprovincialis* waste as the sole food source was evaluated in terms of survivorship and somatic growth performances over four-month experiment.

Results

At the end of the experiment, both *H. tubulosa* and *H. polii* showed high survival, respectively of 94 % and 92 %. Instead, in terms of growth performances, high differences emerged between the two species and the two size classes tested. *H. tubulosa* showed higher growth than *H. polii*, respectively of 6.07 % and -25.37 %. When comparing growth performances among size classes, the highest SGR % was achieved in small *H. tubulosa* specimens (0.09 % day-1), which resulted significantly higher than the value obtained in large ones (0.01 % day-1) (Fig. 1). Both these values, however, were higher than those achieved in *H. polii*, which gained negative somatic growth in both size classes (SGR of -0.26 % day-1 and -0.45 % day-1 respectively for small and large specimens).

Discussion

The present study evaluated for the first time on European species the potential of integrated aquaculture between sea cucumbers and mussels. This polyculture is potentially able to increase energy-use efficiency inside mussel farms, promoting productivity increase and rearing diversification with new commercially important species. Furthermore, these results clearly indicate the different compatibility of *H. tubulosa* and *H. polii* in polyculture with mussels, highlighting that these two sea cucumbers have different feeding behaviors and culture requirements. In fact, Boncagni et al. (2019) observed that *H. tubulosa* and *H. polii* present species-specific use of the same resources that would allow their co-existence in the same benthic ecosystems. This partial degree of niche segregation, which could be related to the differences in digestive physiology (e.g in terms of intestinal microbiota) and feeding specialization in microhabitat (different sediment layers), potentially explained the better suitability of *H. tubulosa* for polyculture with Mediterranean mussel *M. galloprovincialis*. However, we hypothesize that the high rearing density of *H. tubulosa* in the present study could have mitigated its growth performances. Hence future investigations are needed to define the optimal rearing density of this sea cucumber species, to be adopted as extractive species in association with commercial farms of *M. galloprovincialis*.

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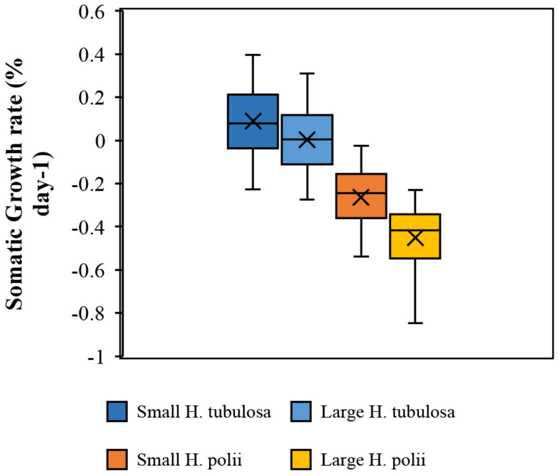

Figure 1. Somatic growth rate (SGR %) of small and large specimens of H. tubulosa and H. polii over four-month experiment under off-shore mussel farm

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CELL CULTURE TEST SYSTEMS FROM FISH FOR VARIOUS APPLICATION IN AQUACULTURE RESEARCH

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Introduction

As the global human population grows and wild fish stocks decline, fish farming and production has become the fastest growing area of food production, achieving a steady growth of 8.4% since the 1970s (FAO 2016). For this, a profound understanding of fish physiology is crucial for successful and sustainable aquaculture production, especially in terms of animal welfare. To achieve this, fishes are presently the subject of a large number of studies where they are exemplarily exposed to different abiotic parameters in order to investigate their physiological stress responses to temperature change (aspect of global warming) or environmental pollution (aspect of ecotoxicology). Furthermore, fishes are also used in animal experiments for the understanding of diseases and tests of medicinal treatment. Already in 1962, the first fish cell line was established by Wolf & Quimby to study these factors. Since then, the number of fish cell lines has increased to about 900 cell lines, reflecting the scientific interest in these species (https://web.expasy.org/cellosaurus/). Although the possibilities of in vitro models have greatly improved, live fishes remain a popular model for animal research. In the statistic of animal experiments, they are second, being surpassed only by rodents and accounting for 14% of all animal experiments in the United Kingdom and 16% in Germany. Altogether, the number of fishes used in research has increased by 54% from 2015 to 2019 (https://speakingofresearch.com/, BMEL.de).

It has already been shown that cell lines can be useful tools for studying the effects of various biotic and abiotic factors, such as temperature changes (Schäfer et al., 2021) and virus-host pathogen interactions and pathogenesis (Noguera et al., 2017). Now, the goal of our work is to establish and to evaluate cell lines from several economically important fishes such as Atlantic sturgeon (*Acipenser oxyrinchus*), rainbow trout (*Oncorhynchus mykiss*), whitefish (*Coregonus mareana*), and Atlantic salmon (*Salmo salar*) as potential in vitro models for fundamental aquaculture-related research.

Methods

Cells were isolated from different fish species and tissues by dissection with simultaneous enzymatic digestion (e.g. trypsin or collagenase). Specific cultivation environments were applied for each fish cell line. Hereby, temperatures below 22°C were usually taken for cold water species, while warm water species cell lines were treated with temperatures of 25°C or above.

After the successful establishment, fish cells exhibited unhindered growth/proliferation due to their telomerase activity, so that their cells can be produced in very high numbers for a wide variety of studies.

Results

We have succeeded in establishing well proliferating cell populations from a variety of fish species that have been passaged and propagated over several years. Hereby the cells exhibited a uniform cell morphology with increasing numbers of passages. Tests on the Atlantic sturgeon and whitefish cell lines demonstrated differences in the physiological reactions in between the species, exemplarily in the responses to both temperature changes and environmental toxins (Grunow et al., 2021).

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Cell isolation of fish larvae from several species including trout, salmon, whitefish, sturgeon, pikeperch, and zebrafish resulted in spontaneously contracting cell (SCC) aggregates (Grunow et al., 2011, Grunow et al., 2015). Characterization of the cells revealed fully developed cardiac muscle and endothelial and fibroblast cells that were electrically and mechanically connected (Grunow et al., 2012, Grunow et al., 2011). The autonomic contraction of SCC is stable over time and resembled the heart beat frequency of fish. This in vitro model is suitable to study cardiac specific fish viruses, such as Infection Salmon Anemia (ISA) or Salmonid Alpha Virus (SAV). Supportively, analysis of viral infection by qPCR revealed that SCCs can be infected with different SAV (F93-125, MS 4640, and F97-220) and ISAV (ISA 390-98) isolates (Noguera et al., 2017, Noguera et al., 2021).

Discussion

Fishes present an outstanding biodiversity possessing wide ranges of evolutionary adaptions to various environmental circumstances. Consequently, species possibly react very differently to external influences. The establishment of cell lines from different fish species is therefore important to detect species-specific peculiarities. In the recent years, we demonstrated that in vitro models are a bioethical and cost-effective tool to study the effects of changing climatic factors or the interaction between host and viral pathogens. Our future goal is to establish stable cell lines from a broad taxonomic range of fish species and to answer the question in which ways suitable in vitro tools can replace in vivo models.

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GILL HEALTH IN MARINE FISH AND BENEFIT OF FUNCTIONAL FEED AS PART OF THE HOLISTIC MANAGEMENT STRATEGY

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The gills of teleost fish are essential for the gas exchange and play an important role in regulation of mineral ions and pH of blood. Gills are also the primary organ of excretion of nitrogenous waste. As one of the main mucosal immune barriers in fish, gills are the first line of defense against all kinds of biotic and abiotic insults. Insulted gills often manifest epithelial hyperplasia/hypertrophy, inflammation, degenerative changes, and circulatory disturbance. When the insults are persistent, pathologies can exacerbate to clinical gill diseases with macroscopic lesions, which indicates that gill function is seriously compromised. Impaired gill function has broad impacts on the fish health including loss of appetite, insufficient oxygen exchange, low-stress tolerance, susceptibility to the secondary infections, and increased mortality.

In marine environment, most of fish production occurs in open or semi-open systems. Gill diseases associated with infectious and non-infectious agents are one of the major health challenges in the farmed fish worldwide.

Similar to complex gill disease (CGD) observed in farmed Atlantic salmon during the seawater phase, the gill disease with multifactorial etiology and non-specific histopathological changes is commonly seen in marine fish in the sea cages. As part of the complex gill disease, infestations of hematophagous monogenean gill parasites, *Sparicotyle chrysophrii, Sciaenacotyle panceri,* and *Zeuxapta seriola*, in gilthead sea bream (*Sparus aurata*), meagre (*Argyrosomus regius*), and yellowtail kingfish (*Seriola lalandi*), respectively, are considered as one of the biggest obstacles for increasing their production in the sea. Histopathological lesions and mechanical blockage of water flow and gas exchange in the gills cause the severe detrimental impact on fish health. If fish are left untreated, the parasites cause not only growth impairment, but also mortality associated with anemia and secondary infection.

Formalin and praziquantel are the most common treatments for gill parasitic diseases. These treatments are not only expensive and labor dependent but also highly stressful for the fish. The secondary effects on development of resistance to anti-parasitic treatment and environmental pollution have raised concerns in the public. Furthermore, all these treatments are not able to prevent the re-infections.

Elimination of above-mentioned gill diseases in the open system is “Mission impossible”. The exchange of parasitic agents between the wild and farmed fish is unavoidable. Mitigation efforts should focus on holistic preventative control strategy. In addition to optimizing the farming practices and environmental hygiene, functional feed concept should be part of the control strategy.

With aim to combat gill diseases in marine species, an extensive field experiment was executed, trials with monthly sampling in two marine fish species, Sea bream (45-155 g) and meagre (75-600g), and in two Mediterranean countries during 2020-2021. Blood parameters, gill histology and growth performance were evaluated, which provided an insight on how growth and welfare were affected by compromised gills and the beneficial effects of functional nutrition. Holistic control strategy with incorporation of functional feed is a potential solution for control of gills diseases.

INCLUSION OF BLACK SOLDIER FLY MEALS IN PACIFIC WHITE SHRIMP DIETS: EFFECTS ON GROWTH & HEALTH PERFORMANCES

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Introduction

For several years, feed manufacturers are searching for new sources of proteins to respond to the growth of the sector and the challenges of sustainable development. At the same time, more than 30% of the world agricultural production is wasted (unsold or expired products, by-products of agri-food industries). Recommended by the Food and Agriculture Organization of the United Nations (FAO), insects make it possible to valorize food waste (bioconversion). The black soldier fly (*Hermetia illucens*) represents a particularly adapted resource to feed fish or shrimps because it is natural, safe, sustainably produced and has a good nutritional quality and potential positive effects on the health.

The objective of the study carried out in the facilities of IMAQUA (Merelbeke, Belgium) between December 2021 and January 2022 is to evaluate zootechnical performances and health protection on juvenile shrimps (*Penaeus vannamei*) by replacing fishmeal with defatted black soldier fly meal (IM) sourced from different Insect Bioconversion Units (IBU) in comparison to a conventional feed (CTRL).

Materials and methods

Three IM produced by three IBU were incorporated in pelleted feeds as replacement material from 27% to 66% of the fishmeal compared to the CTRL. These feeds have been given to 720 Pacific white shrimps from 0.93 grams to 9.86 grams (42 days of trial). The shrimps were divided per group and in triplicates (60 shrimps per tank). The feed distribution was done automatically 6 times a day. The groups of shrimps received the respective diets at the predetermined percentages of their initial mean body weight and expected daily growth. This was adjusted daily according to the expected growth and observed mortality and feed consumption per group.

After the growth trial, 30 shrimps per treatment were transferred to individual tanks for an Acute Hepatopancreatic Necrosis Disease (AHPND) challenge (10 days). The inoculation of the bacterial agent was done by immersion. Shrimps were then monitored twice a day for clinical signs of disease and mortality.

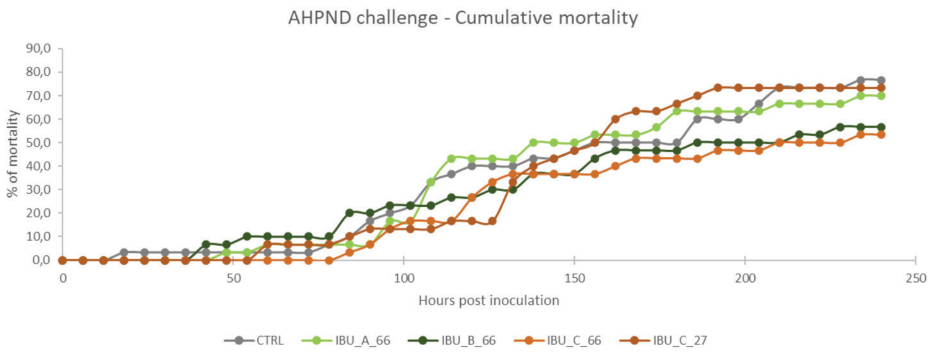

Figure 1: Graphic overview of the results collected during the AHPND challenge

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Results

All results of the growth phase were positive, and the CTRL was validated. Although not significant, an increased final weight (and related weight gain) was observed in all treatments where fishmeal was replaced with IM in comparison to the CTRL. The AHNPD challenge showed expected results for the CTRL with a final mortality of 76.7%. Although not significant, there was a clear trend of decreased mortality at the highest IM inclusion rates compared to the CTRL (Figure 1). Also, onset of observed mortality in all IM groups have been delayed in comparison to the CTRL.

Conclusion

These results suggest 1) a positive impact on growth performance in shrimp at all inclusion rates, and 2) no significant difference between the 3 IBU. It therefore seems possible to replace a significant portion (up to 66%) of fishmeal by IM as this ingredient meets the nutritional needs of shrimps as mentioned by other authors (Mastoraki *et al.,* 2020) who did similar studies on juvenile Baltic prawns. Other studies that used insect meal as fishmeal replacement in shrimp feeds observed the same type of results with a high palatability among diets containing BSF meal (Cummins *et al.,* 2017) and no effects on color and firmness of the shrimp fed with different proportions of mealworm meal (Panini *et al.,* 2017).

This study suggests that although the insect sector is relatively recent, good quality IM (nutritional properties, functionalities) could already be achieved and industrially produced by certain suppliers, using different farming and processing technologies.

As well, reinforcing previous observed tendencies, results obtained suggest that inclusion of IM under certain conditions has potential to improve shrimp resistance to certain pathogenic agents (AHNPD). Further research will be necessary to have a complete picture on how to correctly produce and apply IM in feeds for protecting shrimp against AHNPD. Three components of IM have been identified in the literature as having a potential on health improvements: chitin, lauric acid and antimicrobial peptides (Gasco *et al.,* 2018).

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Acknowledgement

Particular thanks to VEOLIA and IMAQUA for their contribution and cooperation.

BIOFILM-BASED PRODUCTION OF *Tetraselmis suecica* AND ITS FORMULATIONS AS LIVE FEEDS FOR HATCHERIES

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Current microalgae production systems are mainly based on suspended cultures using large volumes of water such as in raceway ponds and photobioreactors. The relatively low cell density or biomass concentration observed within these planktonic-based systems requires energetic expenses for culture agitation, and for biomass harvesting and dewatering. Therefore, an innovative technology has been developed and patented by the inalve company to further enhance productivity at reduced costs, overcoming the drawbacks of conventional systems. Created in 2016, inalve produces microalgae and formulated products used as live feeds for the aquaculture industry. Indeed, microalgae are required during the first-feeding of fish larvae, either for direct consumption or indirectly as food for living preys. Based on Rotating Algal Biofilm (RAB) cultivation, the process does not spend energy to mix the culture, and harvesting is simply carried out by scraping the attached biomass with minimal energy demand. This gentle harvesting procedure allows to produce a concentrated algae paste (15-20% DW) with high cell viability and long shelf-life conservation (>50% alive cells after one month at 4 °C). Therefore, the poster will describe the advantages of the microalgal biofilm production system developed by inalve and the use of the produce microalgae and formulated products by marine hatcheries as feedstock for rotifers, copepods and shrimp larvae.

EFFECT OF SULPHUR ON SELENIUM ACCUMULATION IN *Nannochloropsis oceanica*

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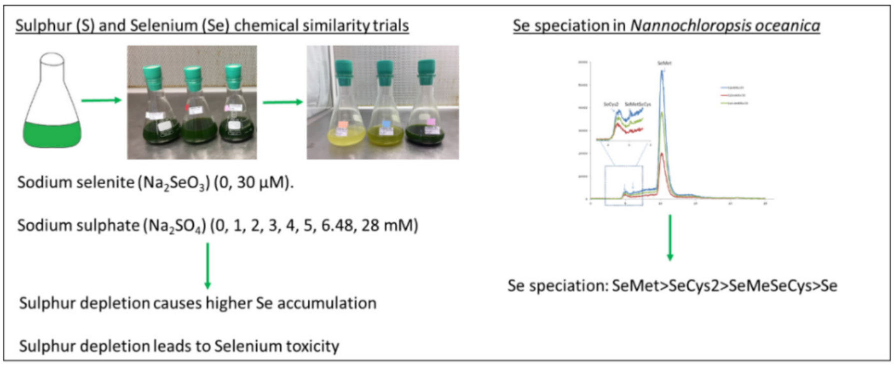
Sulphur (S) and selenium (Se) are chemically similar. Once Se is taken up, it substitutes S in S-containing amino acids. This study investigated the effect of S on selenite accumulation in the microalga *Nannochloropsis oceanica*. [0-28 mM] S concentrations and selenite concentrations of 0 and 30 μM were tested. S concentrations of ≤ 3 mM led to decreased cell growth whereas cultures with ≥ 4 mM were not growth limited. Se accumulation increased up to 8-fold when using S ≤ 2 mM and decreased with S 28 mM. The average relative abundance of organic Se species was selenomethionine (SeMet) 98.2%, selenocystine ($SeCys_2$) 1.4% and selenomethyl selenocysteine (SeMeSeCys) 0.4%. Total fatty acids were not affected by S limitation or Se presence. This is the first study on the effect of S on selenite accumulation, organic Se speciation of *N. oceanica* and its potential as an organic Se-enriched food/feed ingredient.

Graphical abstract


Sulphur (S) and Selenium (Se) chemical similarity trials

Sodium selenite (Na_2SeO_3) (0, 30 µM).

Sodium sulphate (Na_2SO_4) (0, 1, 2, 3, 4, 5, 6.48, 28 mM)

Sulphur depletion causes higher Se accumulation

Sulphur depletion leads to Selenium toxicity

Se speciation in *Nannochloropsis oceanica*

Se speciation: SeMet>SeCys2>SeMeSeCys>Se

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Results

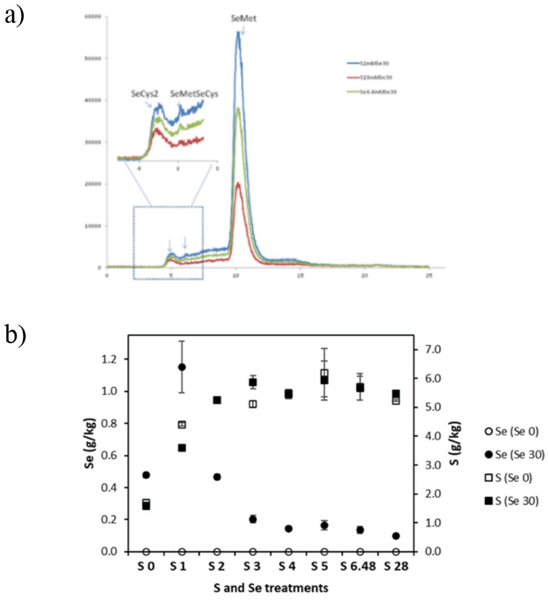

Fig. 1. a) Superimposed chromatograms of the Selenium (Se) speciation within the three Sulphur (S) treatments (S 2, 6.48, 28 mM) containing Se (30 µM) and b) S and Se accumulation in microalgal cultures after twelve days of batch cultivation (n=3, n=9 for controls 6.48 Se 0 and 6.48 Se 30, respectively). *N. oceanica* cultures were exposed to different S concentrations {0-28 mM}, and were either untreated or treated with selenium (30 µM), supplemented in the form of sodium selenite

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PRO HEALTH FEED TO MITIGATE SPARICOTYLOSIS EFFECTS IN CAGED GILTHEAD SEABREAM (*Sparus aurata*): PRELIMINARY RESULTS

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Introduction

Sparicotylosis is a parasitic disease caused by *Sparicotyle chrysophrii*, a blood-feeding gill fluke of gilthead sea bream *Sparus aurata* currently considered as one of the most relevant limiting factors in sea bream Mediterranean mariculture. *S. chrysophrii* has a direct transmission between fish, producing eggs able to spread in the water attaching to the gills host or entangling sea cages nets and hatching the oncomiracidia, ciliated infective larval stage able to actively reach the host. Life cycle of the parasite is strongly influenced by environmental factors, *in primis* temperature. *S. chrysophrii* can be responsible for worsening growth performance and, in the case of massive infections in more susceptible fish, even mortality. Currently, there is a lack of therapeutics both effective and cost-effective, environmentally friendly and authorized in the European aquaculture. In this context, the development of feasible and sustainable control tools such as the use of specific functional feeds is deemed an optimal option as they could combine direct effects on the parasite with positive effects on host immunity and growth, always when administered in the context of proper health management practices. Among the additives identified for the formulation of functional feeds with anti-parasitic action, caprylic acid is considered a promising compound and has been proven to be effective at a concentration of 200 mg/kg b.w. in reducing quantitative parameters of *S. chrysophrii* infection (Rigos et al., 2013) mainly if integrated with MOS and iron (Rigos et al., 2016) and when administered since the early stages of infection. Therefore, in the framework of the EU H2020 Project NewTechAqua (https://www.newtechaqua.eu) and following the results of preliminary lab feeding tests, two functional feeds (Feed A and Feed B) with a mix based on herbs extracts, essential oils, fatty acids (caprylic acid, Feed B double dosage than Feed A), complex of vitamins B (originated from yeast - *Saccharomyces cerevisiae*), iron (chelate of amino-acids) and zinc (chelate of amino-acids) were selected for a field trial on caged sea bream in order to verify their effects on Sparicotylosis mitigation and growth performance.

Material and methods

In January 2021, 60000 gilthead seabream juveniles (mean weight 3g) were seeded in 6 cages (9x5x5m) and divided into 3 experimental groups: 2 cages fed with Feed A, 2 cages with Feed B and 2 cages with standard commercial feeds considered as control group. Samplings were carried out monthly from February to November 2021 collecting hematocrit and biometrical (length, weight) and environmental (oxygen and temperature) data. Parasitological and histological analyses were performed in order to assess quantitative infection data (prevalence, mean intensity, MI, and mean abundance, MA) and related gill pathology during the whole trial. A generalized linear model for the mean abundance of *Sparicotyle* was developed, assuming a negative binomial distribution for the parasite count in each cage. Such model extends the Poisson one, which is commonly used for count data, allowing to manage the issue of overdispersion (i.e. a variance notably larger than the mean) detected in the data. After including all the biometrical and environmental information in the starting model, the best combination of variables independently associated with the abundance of parasites was selected via forward stepwise procedure. Following a similar procedure, a linear model for the average fish weight in the cages was fitted applying the logarithmic transform to fix heteroscedasticity issues, thus leading to a log-normal model.

	coefficient	exponential	standard error	95% C.I. (exp)	p-value
intercept	5.724	-	-	-	-
temperature (°C)	0.090	1.095	0.025	(1.039; 1.153)	2.91×10^{-4}
age (days)	0.007	1.007	0.002	(1.004; 1.011)	2.99×10^{-5}
condition index (g/cm^3 × 100)	-2.203	0.110	0.981	(0.013; 0.875)	2.48×10^{-2}
feed B (yes/no)	-0.333	0.716	0.125	(0.563; 0.915)	7.46×10^{-3}

Table 1: estimated coefficients, together with their exponential (for interpretation purpose), standard error, 95% confidence interval (of the exponential) and p-value for the model for the mean abundance of *Sparicotyle*.

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Results and Discussion

During the trial the MI values ranged from 1.09 in K4 (March) to 8.05 in K1 (July), and the MA values from 0.12 in K1 and K4 (March) to 7.70 in K1 (July). The peak of MI and MA values were observed in July (5.10 and 4.54), followed by August (3.04 and 2.28) when temperatures reached the highest values of 23.9 and 25.8°C, respectively, confirming the role of temperature in parasite load modulation.

Four variables were selected for the negative binomial model, which proved to be adequate according to the goodness-of-fit test (p-value = 0.218), with a pseudo-R^2 of 0.75. According to the coefficients estimated for such model (reported in Table 1), a direct proportionality of the parasite abundance with the water temperature and the time elapsed from the trial beginning is confirmed, as well as its inverse relation with the fish size in terms of condition index. Furthermore, while no significant difference between Feed A (single dose of CA) results and those of the control group has been detected, Feed B (double dose of CA) proved to be a protecting factor reducing the mean abundance of *Sparicotyle* by about 38.4% with respect to the control feed and Feed A at the same temperature, age, and condition index.

The log-normal model fitted almost perfectly the weight data, explaining 99.1% of the variability; not surprisingly, the model resulted adequate according to the test, with a p-value close to 1. The feed not being included in the model after the stepwise forward procedure indicates that there is no significant difference between the three feeds in terms of fish weight when controlling for time elapsed, hematocrit, oxygen concentration, water temperature and mean intensity of the infection, which are the variables selected (estimates not reported here). Focusing on the intensity, which is highly correlated with the abundance (r = 0.985), this variable was confirmed to be a risk factor for weight loss, reducing the average weight in the cage of about 6.3% for each additional parasite per infected fish (for fixed values taken by the other quantities).

In conclusion, we confirmed the caprylic acid's anthelmintic effectiveness when administered at the proper dosage in limiting the course of infection and mitigating the effects of Sparicotylosis in farmed gilthead seabream when administered since the introduction of fish into cages and before high levels of parasitic infection are established. Feed B could therefore represent an effective prevention tool to reduce the impact of Sparicotylosis on farmed gilthead seabream, albeit further steps would require its administration during a normal production cycle, to confirm these promising experimental results. The preliminary results achieved in this study will be implemented by further analyses to associate the collected data with histopathological studies on gill environment during Sparicotylosis occurrence.

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BENCH TOP VISUAL HAB MONITORING: SUPPORTING CLIMATE RESILIENT AQUACULTURE IN THE ATLANTIC AREA

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Introduction

Harmful algae blooms (HABs) are characterized by a massive proliferation of phytoplankton organisms, which provide a myriad of adverse effects such as large-scale marine mortality, economic impacts in coastal regions and consequences to aquaculture industries. Climate change has affected HAB frequency and severity on a global scale. In this scenario, machine learning may be an invaluable tool in helping society adapt to the effects of climate change through reliable HAB monitoring and early detection.

Plankton data sets are usually imbalanced and reflect natural differences within the environment. For minority classes, there may not be enough data to properly represent this variability, preventing AI models from gaining a full understanding of these classes (Kerr et al., 2020). The present work employs state-of-the-art Deep Learning (DL) models to support HAB monitoring applications within the Atlantic area.

Materials and Methods

A unified benchmark database covering publicly available phytoplankton images has been built through a data integration pipeline (Guterres et al., 2021) considering target phytoplankton genera from Integrated Multi-Trophic Aquaculture (IMTA) farms from Brazil, South Africa and Scotland. Classic convolutional neural networks architectures are trained for phytoplankton classification. Best individual models serve as a baseline for investigating state-of-the-art methods for class imbalance classification of target phytoplankton organisms. The following approaches have been evaluated to best support climate resilient solutions on HAB monitoring:

- Two-phase Learning (2PL) combines the Random Under Sampling technique with transfer learning. The model is first pre-trained using threshold data and then fine-tuned using original unbalanced datasets (Buda et al., 2018).
- Dynamic Sampling (DS) dynamically changes the class distribution of the training samples. Initially, the number of samples of each class equals the average number of samples. For every other iteration, the number of samples of each class is calculated based on the F1-Score from the previous training round (Johnson et al., 2019).
- Threshold Moving may be implemented on already trained models to improve classification results. It adjusts the decision threshold of a classifier during the test phase. Considering neural networks estimate Bayesian *a posteriori probability*, the output y for class i implicitly corresponds to for a given datapoint x. The correct class probabilities can be obtained by dividing the network output for each class by its estimated prior probability (Buda et al., 2018).
- Deep collaborative models (ensemble) may harness the limited understanding of individual models to provide a collective and more accurate classification specially for minority classes. It is a heterogeneous ensemble of DL models which grants a substantial performance improvement regarding other state-of-the-art approaches (Buda et al., 2018).

Results and Discussions

MobileNetV2 was selected as baseline model for further DL modeling since they provided best results among other architectures (NasNet, Resnet and VGG16). They are also targeted towards embedded and resource constrained environments. Table 1 depicts performance results within state-of-the-art approaches for phytoplankton classification.

All investigated methods have improved classification performance compared to the baseline architecture. Collaborative deep learning model showed promising results. It enabled the combination of other state-of-the-art approaches towards reliable phytoplankton and HAB monitoring. Threshold moving has provided outstanding performance compared to other investigated approaches.

Conclusions

The present work investigated state-of-the-art approaches to class imbalance classification, considering target phytoplankton organisms within the Atlantic area. Deep collaborative models and threshold moving may be key methods towards climate resilient solutions for HAB monitoring since they can be employed upon latest DL models and architectures.

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Table 1 - Performance results within state-of-the-art methods for classification of target phytoplankton genera in IMTA applications.

Method	Recall	Precision	F1-Score	Model Size
None (Baseline)	0.75	0.78	0.75	29.1MB
DS	0.88	0.77	0.82	23MB
2PL	0.84	0.91	0.87	54.8MB
Ensemble (DS + 2PL)	0.87	0.94	0.89	50MB
Threshold Moving (Ensemble)	0.88	0.94	0.91	50MB

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EFFECTS OF TEMPERATURE, SALINITY AND DIET ON GROWTH, SURVIVAL AND FATTY ACID COMPOSITION OF THE RAGWORM *Hediste diversicolor* (OF MÜLLER, 1776) (ANNELIDA: NEREIDAE)

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Introduction

The common ragworm *Hediste diversicolor* is an omnivorous, burrowing polychaete with a broad tolerance to environmental conditions such as temperature, salinity and oxygen levels. This nutritious annelid has received a great deal of attention lately for its potential as an extractive species in IMTA systems to recapture high-value compounds from fed aquaculture particulate side streams (sludge) and its suitability for subsequent utilisation as a novel, omega-3 rich aquafeed ingredient (Wang et al., 2019). It has recently been shown that this species has the capacity for endogenous production of omega-3 long-chain polyunsaturated fatty acids (Kabeya, et al., 2020), however it is yet not understood how environmental cues affects this ability. We conducted two sets of experiments to assess the combined effects of diet, temperature and salinity on growth, survival and total body fatty acid composition in *H. diversicolor* juveniles.

Materials and Methods

Polychaetes (*H. diversicolor*) were collected from Leangen Bay, Trondheim, Norway (63°26′24.5″N, 10°28′27.7″E). To investigate the effects of diet and temperature on growth, survival and fatty acid (FA) composition of *H. diversicolor*, worms were fed mixes of solid biogas digestate (SBD) and salmon aquaculture sludge (SS) along a 4-step feed gradient ranging from pure SBD to pure SS, and a 5-step temperature gradient ranging from 5.8 to 17.1 °C, for 15 days, using fish feed as a control (Experiment 1). A second experiment was conducted to investigate the effects of salinity and temperature on the same variables. Here, the worms were fed the diet which yielded the highest growth rates in the first experiment (33:66 % SS:SBD) along 5-step salinity and temperature gradients ranging from 5 to 40 ppt and 7.7 to 17.9 °C, respectively, for a duration of 28 days. In both experiments, worms were fed isonitrogenous diets equalling 30 % of the worms' total body nitrogen per day (Wang et al., 2019). Both experiments were conducted in a temperature gradient table (modified after Thomas et al. (1963)) using a 18h:6h light:dark cycle. Worms (n=7-8) were stocked in glass beakers (800 mL) containing an eight cm thick layer of sand and sand- and bagfiltered (1 μm) seawater from the Trondheim fjord collected at 60 m depth. The worms were allowed to evacuate their guts in clean seawater for minimum 4 hours before each sampling and weighing. Water exchange and feeding was conducted every second day. Data analyses were performed using the inbuild statistical package of SigmaPlot v. 14.5.

Results and Discussion

In the first experiment, the highest specific growth rate (SGR) was found for worms fed a 33:66 % mix of SS:SBD (SGR= 0.025) at 14.7 **°C**. Growth was found to be significantly influenced by temperature (two-way ANOVA; $p<0.001$) and not by diet (two-way ANOVA; $p>0.05$) (Figure 1A). Growth was only positively related to temperature up till 14.7 °C, whereafter SGR declined. The proportions of SS and SBD in the diet showed no significant effects on SGR within temperatures. Survival was high for all treatments combined (88±13 %), and both temperature and diet had significant effects on survival (two-way ANOVA; $p<0.001$ and $p>0.05$, respectively); mortality increased with increasing temperature, whereas feed type did not have such a pronounced, yet significant, effect on survival. In the second experiment, the highest specific growth rates (≤0.015) were found for worms maintained at high temperatures (≥14.3 °C) and high salinities (≥35 psu) (Figure 1B). Survival averaged 80.5±16.2 % for all treatments and was, opposed to growth rate, not significantly affected by neither salinity (One-Way ANOVA, Holm-Sidak *post hoc*; $p>0.05$) nor temperature (Kruskal-Wallis One Way Analysis of Variance on Ranks; p= 0.380).

No temperature- nor salinity-driven segregation patterns could be identified in the FA profiles of the worms, however a clear diet-driven segregation was found between worms fed lipid-rich fish feed (control) and lipid-poor SS and SBD diets (data not shown). Hence, our results indicate that the major influence on fatty acid composition in *H. diversicolor* is diet. We here demonstrated that short-term (≤4 weeks) alterations of environmental parameters have neglectable effects on the lipid profile in wild caught *H. diversicolor* juveniles (Villena-Rodríguez, A. et al., *in prep*, data not shown).

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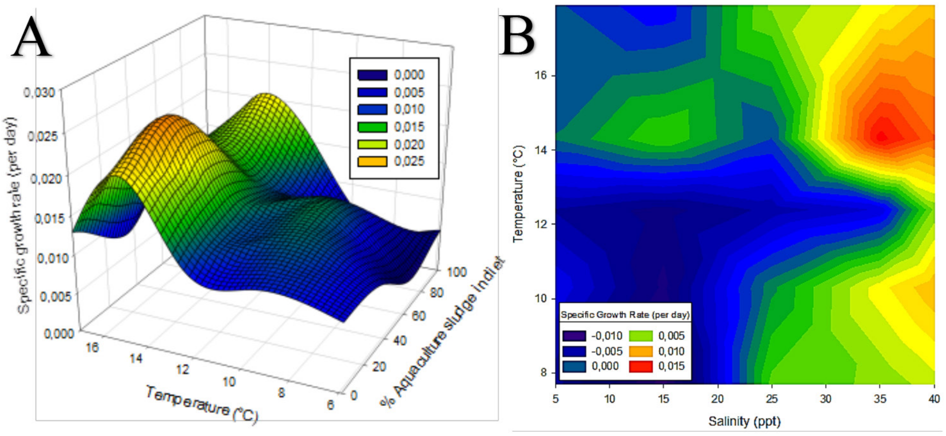

Figure 1. Specific growth rate (SGR) for *H. diversicolor* fed different ratios of solid biogas digestate (SBD) and salmon sludge (SS) at different temperatures over a 15-day period (A), and *H. diversicolor* fed the same diet at different temperatures and salinities over the course of 28 days (B).

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PHYSICAL PLASMA FOR DECONTAMINATION IN AQUACULTURE

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Clean water is an essential good although its preservation and supply can be very challenging. This includes the sustainable management in production processes and especially aquacultures. An efficient remediation of aquacultural water should reduce freshwater consumption and thereby costs as well as ensures healthy products. The utilization of physical plasma has been proven as efficient method for water remediation with inherent advantages (Malik et al. 2001). In particular, the formation of reactive oxygen and nitrogen species (such as ozone, hydrogen peroxide, nitrite, nitrate and hydroxyl radicals) provides effective means for the inactivation of microorganisms and the degradation of pollutants (Lu et al. 2016; Banaschik et al. 2018; Hahn et al. 2019). Respective processes are supported by the emission of electromagnetic radiation i.e. ultraviolet light and strong electric fields.

In this regard, plasma can be applied for the inactivation of microorganisms and viruses. An inductively-limited discharge (Schmidt et al. 2017) was used for the treatment of the T4-bacteriophage (virus, DSM4505; host microorganism: *E. coli* DSM613) and the multidrug-resistent microorganisms *Klebsiella pneumoniae* (DSM109397) or methicillin-resistent *Staphylococcus aureus* (MRSA; DSM 18827). The examined test objects were inactivated by up to six orders of magnitude depending on the exposure time after plasma treatment. In addition, the pollutant bisphenol A was transformed by 34% in preliminary experiments. Interestingly, reactive species that are provided by plasma can be preserved by freezing the plasma-treated liquid. With respect to aquaculture this might offer another possibility for the extended shelf life of fish or shrimp by the inhibition of microbial growth for the storage on frozen plasma-treated water i.e. ice.

Thus, plasma is suitable for the removal of microbial and chemical contaminants and thereby improves water quality and production processes in aquaculture.

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REAL TIME OBSERVATION OF MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* PREDATION BY FLATWORM *Imogine mediterranea* USING VALVE GAPING MUSSEL MONITOR (VGMM)

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Introduction

Mussel *Mytilus galloprovincialis* Lamarck, 1819 is an important commercial mariculture species and powerful bio-indicator commonly used to monitor spatial distributions and temporal trend of pollutants in costal and estuarine areas (Hamer et al., 2008; Pavičić-Hamer et al., 2016). More recently, behavioural markers are applied to assess changes in the health status of mussels and ecosystem in response to different threats, and as a part of biological early warning systems (BEWSs) (Borcherding, 2006). Mussel valve movement (gaping) is widely recognized as an integrative measure of physiological functions such as respiration, feeding and excretion, which can change under stressful conditions in response to a deteriorating environment and as a defence reaction to external stimuli e.g., touching or shading, the sudden approach of a predator (Cameau et al., 2018).

In the summer 2021 and 2022, during monitoring of the intensive ascidian tunicate invasive species *Clavelina oblonga* Herdman, 1880 fouling occurrence at shellfish farm locations in the Lim Bay (NE Adriatic Sea, Croatia), the presence of unusual numerous individuals of the free-living Turbellaria flatworm *Imogine mediterranea* (Galleni, 1976) was determined (Figure 1A,B). It was previously established that *I. mediterranea* feeds on the commercial mussels and oysters (Gammoudi et al., 2017). With following experimental setup (Figure 1C), we answer raised question: Does the flatworm feed only on weakened-death mussel in *C. oblonga* fouling "sods" or can attack healthy mussels also?

Materials and methods

Under laboratory conditions (2 x 40L tanks with aeration and throughflow sea water) 6 acclimated mussels (meat yield 19.4 +/- 0.5%) were connected to Valve Gaping Mussel Monitor (VGMM). Two mussels were used as control and 4 mussels were exposed to 12 flatworm specimens for 3 days (Figure 1C). After acclimation, mussels were monitored 2 days to check normal valve gaping rhythm as a proxy of their filtration and resting activity. Mussel VG was measured by frequency of 0.5 Hz using magnets and Hall sensors fixed on mussel shells connected to microcontroller Arduino Uno and notebook (Figure 1D,E).

Results and Discussion

Analysing and comparing VG results before and during mussel exposure to flatworms, including controls, it was possible to observe mussels VG normal daily rhythm (> 70% time filtrating with valve open > 50%, with 1-5 resting periods valve open < 20% and occurrence < 30%), behaviour changes control vs exposed mussels (stress), the time of flatworm attack, following mussel response during predation and as well as mussel death time (30 h after exposure and 26 h after flatworm attack) (Figure 2).

Flatworm *I. mediterranea* is very likely an extremely opportunistic species i.e., it uses an abundance of food due to the poor mussels condition provoked by the presence of *C. oblonga* fouling, but also can feed on healthy mussels. Such a numerous invasive predator could pose an additional threat to shellfish farms in the Istrian County. According to our field observations and mussel VG results in framework of projects (BlueBio 2020 MuMiFaST and Istria County 2021/2022), there is huge potential of VGMM application for *in*/*ex situ* mussel behaviour and health monitoring as indicator of normal VG behaviour (mussel filtration function), suitable environmental conditions, extreme conditions (CC), fish predation, presence of diseases, including toxic phytoplankton blooms.

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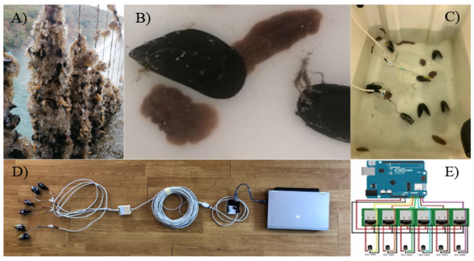

Figure 1. A) Mussel shellfish farm infrastructure threatened by *C. obloga* invasion; B) predation of mussels by flatworm *I. mediterranea*; C) experimental setup; D) VGMM with 6 sensors/mussels; E) Arduino microcontroller and VG Hall sensors connection schema.

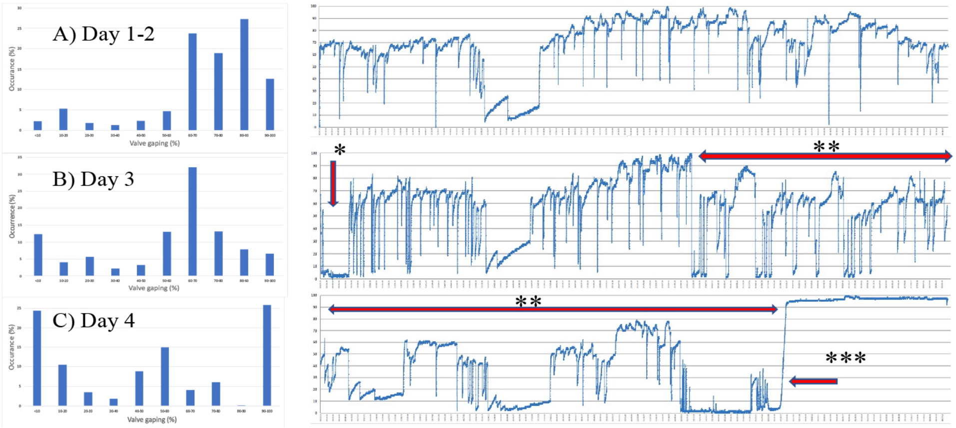

Figure 2. Real time observation of mussel predation by flatworm *I. mediterranea* using valve gaping mussel monitor: A) Day 1-2, mussel acclimation; B) Day 3, beginning of mussel exposure to flatform (*); C) Day 4, first attack and mussel response to inner predation (**) and mussel death (***). Bar graphs represent cumulative daily mussel VG percent occurrence (24 h, 9:00 – 9:00 h).

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CULTURING THE SEA CUCUMBER *Holothuria forskali* IN INTEGRATED MULTITROPHIC AQUACULTURE (IMTA) OFF THE WEST COAST OF IRELAND, A BIOREMEDIATION STUDY

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This study aims to culture sea cucumbers *Holothuria forskali*, carrying out field trials at Lehanagh Pool IMTA Marine Research Site. This involves maintenance of sea cucumber stock, field measurements - monitoring of benthic conditions and nutrient profiles, to develop and test structures for the containment of sea cucumbers under salmon pens. It will provide data on the feasibility and value of sea cucumbers grown under aquaculture structures, how much waste they can eat or remove and assess their use as a food source. Biochemical tracers such as stable isotopes will be used to show a trophic link between sea cucumbers and the fish farm.

Method

Sea cucumbers have been placed in cages on the sea floor and in lantern nets at a low stocking density suspended from a rope 5m above the seafloor near and around salmon pens to trial whether they consume and grow on the waste from the farm above. Evaluation of seeding and harvesting sea cucumbers where fish faeces have built up for site remediation is carried out. This valuable and sustainable site remediation service is followed by sea cucumber utilisation as human food and novel compound resource.

This study was set up at the Marine Institute IMTA research site Lehanagh pool, Bertraghboy Bay, Connemara, County Galway, Ireland. The area is situated on the west coast of Ireland in a sheltered bay. The sediment type is typically muddy and sandy. A small-scale experimental IMTA site where salmon smolts (*Salmo salar)* are reared in sea pens. The site is comprised of 3 polar circle cages (50 m circumference and 8 m deep) at a water depth of 21 m. The site is managed according to organic farm standards, with no prescription medicines or antifoulants used. Sea cucumbers *Holothuria forskali*, Sea urchins *Paracentrotus lividus*, European lobsters *Homarus gammarus*, King Scallops *Pecten maximus* and different species of seaweeds *Alaria* sp. and *Saccharina sp*. are also cultured on this site. Sediment traps have been developed and grab samples taken for Total Organic Carbon, Total Nitrogen and Total Phosphorus and sent for analysis. The salmon are fed a standard commercial organic diet. Water quality is routinely monitored for chemical and biological parameters.

Results

Preliminary results show – Figure 1. Average weights of *H. forskali* at higher stocking density, 100% survival in October in all lines, in March 83% survival in North line and 100% survival in South lines. Animals did well in higher stocking densities. Losses in average weight are possibly due to animal mortalities. Control lines 100% survival in October, 66% survival at March weigh in.

Figure 2. Average weights of *H. forskali* at lower stocking density 100% survival in all lines. In October 50% survival in North lines. Control line 100% survival. The losses in average weights are possibly due to animal mortality.

Figure 3. Temperature data between July 2021 and April 2022 the data is normal for the time of year with a dip in winter time below 9ºC temperatures are beginning to rise now so expect to see further growth in animals.

Conclusions

This may be due to where the animal was in life cycle when caught, no way of knowing age of animal, only by how they look when putting into cages. Weather exposure could be an issue, possibly lack of food over the winter.

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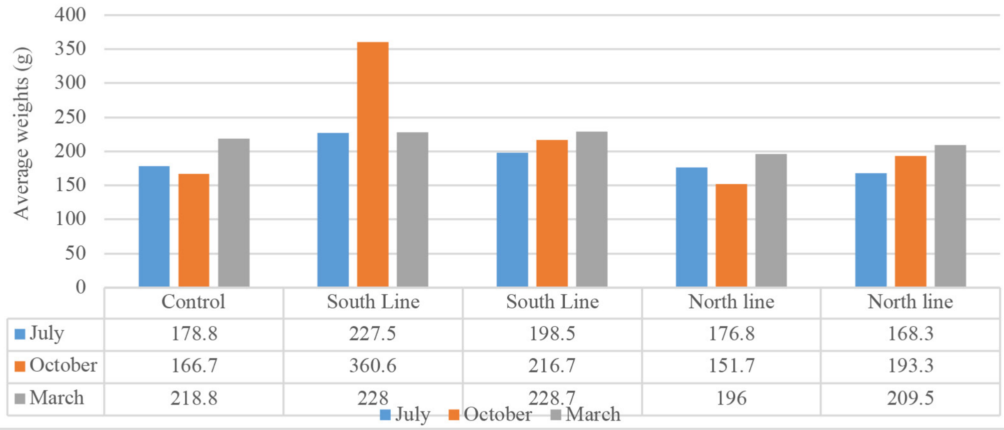
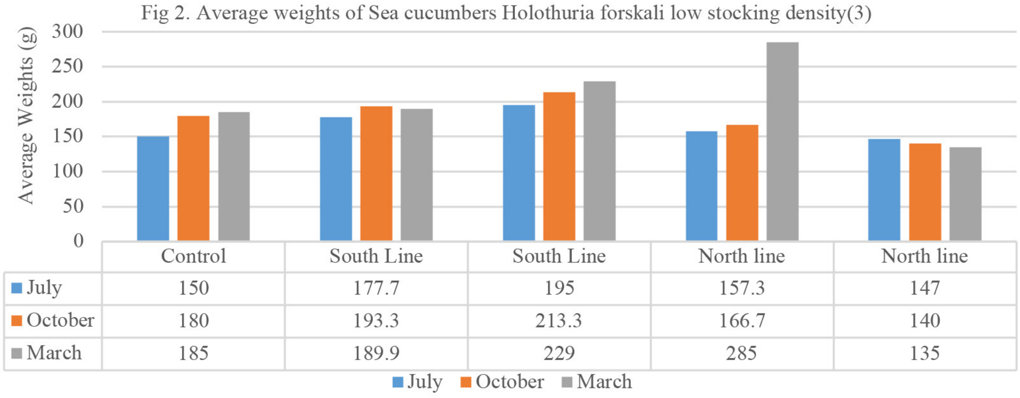
Fig 1. Average weights of Sea cucumbers *Holothuria forskali* high stocking density(6)



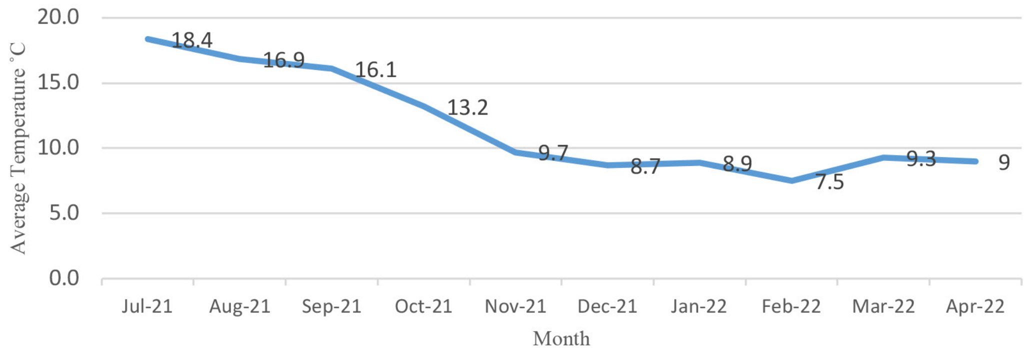
	Control	South Line	South Line	North line	North line
■ July	178.8	227.5	198.5	176.8	168.3
■ October	166.7	360.6	216.7	151.7	193.3
■ March	218.8	228	228.7	196	209.5

Fig 2. Average weights of Sea cucumbers Holothuria forskali low stocking density(3)



	Control	South Line	South Line	North line	North line
■ July	150	177.7	195	157.3	147
■ October	180	193.3	213.3	166.7	140
■ March	185	189.9	229	285	135

Fig.3 Average Temperature per month at Lehanagh Pool site 2021-2022



EFFICACY OF MICROPLASTIC DEPURATION IN TWO COMMERCIAL OYSTER SPECIES FROM THE WEST COAST OF IRELAND

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Introduction

Microplastics (MPs) are recognised as emerging contaminants in the marine environment, and are suspected to affect aquaculture production. Bivalves, such as oysters, are low-trophic organisms that are relatively exposed to microplastics due to their sedentary living and high filter-feeding capacities. There are limited studies focusing on assessing depuration efficacy in commercially grown species, although it is estimated this number will grow given the global efforts to identify and quantify microplastic pollution. This study investigates microplastic depuration efficacy in two commercially grown oyster species, the Pacific oyster (Magallana gigas, n=50) and the European flat oyster (Ostrea edulis, n=50) collected from the West coast of Ireland.

Materials and methods

A depuration set up was run in a temperature-controlled facility (~10 °C). Five depuration treatments were assessed control, 24, 48, 72 and 96-hours (with ten individuals (n=10) per treatment) for each oyster species. A 10% potassium hydroxide (KOH) solution was used to digest the organism (Δ 40 °C for 24-hours). After digestion, the resulting digestate was filtered using a vacuum pump filtration system using glass microfibers filter membranes (Whatman GF/C, Ø = 47 mm, pore size 1.2 μm), under a laminar flow, to minimise airborne contamination. Filters were observed under a stereomicroscope and subsampled for polymer (109 out 539) characterisation using the micro-Fourier Transformed Infrared Spectroscopy. A forensic approach to cross-contamination was applied to minimise all potential particles from clothes and daily use.

Results

Microplastics concentrations prior to depuration in *M. gigas* were 0.6 MP g^{-1} edible tissue and 0.4 MP g^{-1} for *O. edulis*. Significant reduction in MP concentrations after a 96-hour depuration period were measured: 0.2 MP g^{-1} for *M. gigas* and 0.1 MP g^{-1} for *O. edulis,* respectively. Moreover, there was no significant correlation between microplastic concentrations and weight of edible tissue of oysters. Microplastics were mainly fibres, with the main colours being blue, black, red and transparent. Polymer identification from the subsample analysed revealed more than 51.6% of fibres were natural fibres, with the remaining (48.6%) varying synthetic fibres, including Nylon.

Conclusion

The results from this preliminary depuration study showed that increasing depuration time to 96-hour can significantly decrease the concentration of microplastic in edible tissues of selected oyster species. These findings align with other depuration studies showing reduction in microplastics concentrations in bivalves species, however the novelty in our results relies in the fact of using two species, with a large sample size (N=50 per species) and for a longer period of time than conventional laboratory assays (t=96h). These results also contribute to provide information to aquaculture producers and policy makers on how to reduce microplastic exposure in products that are destined for human consumption.

EFFICIENT USE OF RESOURCES IN AQUACULTURE: NEEWA – NETWORK FOR GENERATING ENERGY WITH HYDROPOWER IN EXISTING WATER SYSTEMS

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Current problem and state of knowledge
With the Green Deal, the European Union has committed itself to be climate neutral by 2050. The aim is to no longer release any net greenhouse gas emissions (1). In order to achieve this goal, it is essential to increase the share of renewable energy in final energy consumption. In this way, by 2030, this share of all energy in the EU is expected to increase to 32% (2). These goals are also implemented at national level as part of the climate protection program 2030 (3). In addition to wind power, biomass and solar energy, electricity generation from hydropower offers another option for decentralized electricity generation from a renewable source. In many places, the possibilities of hydropower are probably not exhausted due to legal hurdles or a lack of technical know-how, despite its advantages. The advantages can be seen in particular in 100% clean and base load energy with low production costs. The focus here is on the greatest challenge, the ecological compatibility, which describes the influence on the environment of the power plants, which are mostly installed in natural ecosystems such as rivers. The primary goal is to be able to rule out effects such as endangering fish stocks, as well as negative influences on the surrounding riverbed and its flora. Technological possibilities are insufficiently known, and political education makes expansion more difficult. Technological solutions are also anchored in special machine construction, which excludes series certification and thus results in expensive certification costs for each individual system.

Subject and goals of the project
There is great energy potential in the wastewater from sewage treatment plants, sewer networks and aquacultures, which has not yet been tapped due to a lack of knowledge transfer and, in addition, a lack of research (4). With these systems, energy flows for the realization of hydropower and its advantages (see "Current problem and state of knowledge") can be used profitably, excluding ecosystems. The water used for the processes is withdrawn from the ecosystem in these procedures. In this way, using hydropower within these production systems eliminates the environmental impact, which is the main negative aspect of hydropower.

The aim of this project is, in addition to networking and the exchange of all potential actors from the entire value chain, to bring the participants together from the point of view of knowledge transfer and to train them with regard to opportunities and possibilities. The regular exchange within network meetings and knowledge transfer events provides information on new technologies and the combined use of application processes.

The network is intended to promote the use and further development of existing and new technologies for the use of hydropower in combination with heat exchangers through regular exchange. Another goal of the project is to develop an online decision-making tool and make it available to interested parties through the exchange and central compilation of obstacles and problems of hydropower in order to make a decision on the use of hydropower within your production processes outside the ecosystem enable. In particular in the areas of small-scale hydropower, these are only insufficiently available. These initial findings have already been gained through guided interviews as part of the project. The guide or decision support tool makes it easy to understand the approval process and the necessary steps. The results of the project are published in various places to strengthen a neutral, scientific representation of hydropower in Germany. The aim is to disseminate information about the use of hydropower in anthropogenic water systems in Germany in order to support the exploitation of this clean and sustainable energy potential.

Own preparatory work and previous activities in the field
The Institute for Water and Energy Management, founded in 2015 at the Hof University of Applied Sciences, is an interdisciplinary laboratory of the Engineering Faculty. The institute is currently working on the projects "Development of fully biodegradable growth bodies in aquaponics with the release of nutrient additives for biological water treatment" (ZIM, 2021-2023) and "Network for generating energy with hydropower in existing water systems". (ESF, 2021-2022) by Dr. Harvey Harbach and a team of scientists.

Financing and duration of the project
The planned duration of the project is 18 months. This project is funded by the European Social Fund (ESF) with 417,000 euros.

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EUROPÄISCHE UNION
EUROPÄISCHER SOZIALFONDS
ESF IN BAYERN
WIR INVESTIEREN IN MENSCHEN

IN VITRO ANTIPARASITIC EFFECTS OF SOME HERBAL EXTRACTS ON CILIATA, *Cryptocaryon irritans* THERONTS

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The ciliate protozoan *Cryptocaryon irritans,* which causes white spot disease in fish, is recognized as one of the most dangerous pathogens in cultured marine fish. Since herbal products have less adverse impact on the environment and fish in comparison to chemical treatments, their use in aquaculture against diseases has increased. In the present study, anthelmintic effects of onion (*Allium cepa*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), radish (*Raphanus raphanistrum*) and pomegranate (*Punica granatum*) extract solutions were investigated *in vitro* against *C. irritans* theronts (infective phase). In the experiments, *C. irritans* theronts were subjected to the herbal extract solutions with 100, 50, 25, 12.5, 6.25, 5 and 1% concentrations for 20 minutes. At 100% concentration, 100% cumulative mortality was observed in 1 minutes with pomegranate, radish and garlic extract solutions, whereas corresponding cumulative mortality caused by onion solution was 50% and by ginger solution was 70% in 1 minutes. At the lowest concentration, 1%, tested, 100% cumulative mortality was observed in 20 minutes with pomegranate extract application. No parasite mortality in 20 minutes was observed after tests with onion, ginger and garlic extracts at 1% concentration. *In vitro* results revealed that all solutions had dose and duration dependent antiparasitic effects against *C. irritans* theronts.

OVERVIEW OF THE AQUACULTURE SECTOR AND MSP PROCESSES IN THE MACARONESIAN SEA BASIN

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Introduction

The Macaronesian Sea Basin includes the Portuguese archipelagos of Azores and Madeira and the Spanish archipelago of Canary Islands, which are located in the Central Eastern Atlantic Ocean. In these archipelagos maritime-based activities constitute the backbone of the local economies, with coastal tourism, cruise tourism, shipping and fisheries identified as playing a pivotal economic role. In the last decades, marine aquaculture is assuming a relevant position. The growing trend of cage production for finfish species, such as seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), Amberjacks (*Seriola* spp.) and Meagre (*Argyrosomus regius*) is mainly connected to favourable climatic conditions (e.g., annual stable and high mean sea temperature and short photoperiod variation throughout the year) together with the competition for land space. After the implementation of regional regulations for aquaculture by regional Governments, which include the allocation of marine space, mariculture production is expected to grow in the coming years, On that sense, Madeira published the Plan for Management of Marine Aquaculture of Madeira (2016), the Canary Islands adopted the Regional Plan of Management of Aquaculture (2018) and the Azorean administration is also engaged in the establishment of the sector through the development of a legislative framework for licensing the location of aquaculture production areas and identification of species allowed in the Azores (2016); in all three archipelagos, these initiatives are promoted from tax benefits for investors. The development of aquaculture in Macaronesia entails several concerns about its environmental interactions that needs to be addressed to foster the long-term sustainability of the sector.

Aims

In this contribution we shall present the state of the art on MSP implementation together with the environmental pressures and impacts related to the marine finfish aquaculture in the Macaronesian Sea Basin. Strategic planning and application of technical standards for aquaculture systems to mitigate potential environmental impacts will provide fundamental information to spatial planning processes, ensuring that this and other maritime activities will have access to clean, healthy and productive marine waters.

IT'S GETTING HOT IN HERE; EXPLORING THE EFFECT OF HEAT TREATMENT ON SPAT AND HALF-GROWN OYSTERS (*Crassostrea gigas*)

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Oysters (*Crassostrea gigas*) are in volume after mussels the most important cultivated shellfish species in the EU. In the Netherlands, off-bottom techniques were initiated in 2010 due to high losses by the oyster herpes virus (Ostreid herpesvirus 1; OsHV-1) and predation by the Japanese Oyster drill (*Ocinebrellus inornatus*) on subtidal culture plots. Off-bottom cultivation takes place in the intertidal areas and typically results in oysters with fast growth and high meat quality due to a higher food quality and food availability higher in the water column. While the implementation of such cultivation systems could reduce the mortality caused by the oyster herpes virus and predation of the Japanese Oyster drill, heat exposure of the oysters increased in off-bottom cultivation. Recent summers showed long periods of increased solar radiation compared to 10 years ago. Prolonged sun radiation exposes the oysters in off-bottom systems to temperatures even higher than 40°C. Climate change increases this risk. Heat stress can result in higher mortality amongst oysters and reduce harvest yield for off-bottom cultivation.

In 2020 the European Marine and Fisheries Fund project "*Oyster yield improvement through knowledge transfer and monitoring*" was initiated. The goal of this project is to optimize oyster farming and it should lead to efficiency improvements in sustainable oyster cultivation. As part of this project, a study on the effect of heat exposure on survival of spat and half-grown oysters was carried out. The aim of this study was to investigate the overall mortality rate caused by prolonged exposure to radiation heat of 40 degrees Celsius.

The experiments were carried out at HZ University of Applied Sciences in a climate-regulated room set to a default temperature of 20^{o}C which represents the average Dutch summer water temperatures over the last ten years. During the treatments oysters were exposed to air. Heat radiation was simulated with a heat lamp (EXO Terra Intense Basking Spot PT2140 150-Watt S30).

Two size classes were tested: 1. oyster spat (2n), 13.76 mm (±1.70) was acquired from the Hatchery '*Roem van Yerseke'* and 2. half-grown oysters (3n), 15,6 gr (± 3,03) fresh weight were acquired from the off-bottom location '*Hooge Kraaijer, Delta Ostrea B.V.*' originating from the Hatchery '*France Naissian*'. Triploid oysters were used to prevent spawning, that maybe initiated by a sudden rise in water temperature. Experiments were carried out during winter and spring.

During the experiment, the oysters were exposed to heat radiation for 2 hours a day.

Based on the average inundation of 20% for off-bottom cultivation sites in the Oosterschelde, the Netherlands. Simultaneously, a control was carried out in which oysters were exposed to air at the default temperature of 20^{o}C. After the treatments, the oysters were held in aerated saltwater, set at 20^{o}C. Four consecutive runs were carried out with both oyster spat and half-grown oysters. The number of living oysters was counted before and after treatment. Dead oysters were removed. To simulate a prolonged summer period each run was continued for 12 days or until all oysters died.

An example of survival over time is shown in Figure 1, for the control and exposed (lamp) oysters for the different size classes.

Results are still processed and will be shown and discussed during the presentation.

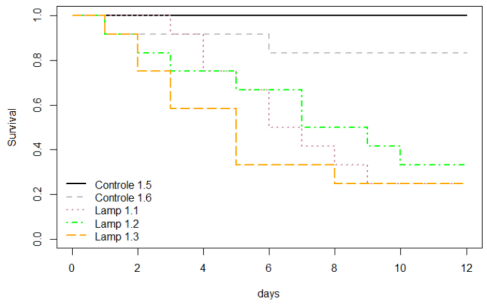

Figure 1, **Example graph of one of the runs;**
Survival over time of half-grown oysters.
(at the time of writing, data processing was ongoing)

COMMERCIALISING YOUR POND: DON’T GET CAUGHT BY LEGAL DISRUPTORS!

Katherine Hawes

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Introduction

Most aquaculture farmers usually work with professional researchers, equipment suppliers, specialists, technology providers, water specialists, feeding companies and other famers to name a few when they set up their farm. This is irrespective of whether the ‘fish’ farm being commercialised is land or ocean based. am aquaculture business.

The question is when it is time throughout that process for either aquaculture professionals or farmers to engage with business experts to manage: your risks, protect your assets and meet your regulatory and compliance requirements. This presentation will take aquaculture professionals and farmers through the commercialization process and outline touchpoints with a business and legal professional.

Aquaculture Farm Start-Ups

Whether you are just starting out or been in business for a while, we have a checklist for you about ensuring your future generations are looked after by you minding your business. We give practical advice on making sure the majority of what you do is protected by both upstream and downstream agreements.

If you employ people, there are a myriad of things today to consider. Some of the key ones are some standard terms and conditions of employment, terminations, management of social media and now of course ensuring you are protected in a pandemic.

If you are engaging with scientists your agreement should specify who is responsible for doing what and who owns what at the end of a trial or experiment. Protecting your intellectual property is paramount to keeping your business safe.

Aquaculture Farm Growth

As you hit critical mass and your business expands, success means your customer expectations change and there is nothing like a great customer service charter and agreement to ensure that both your expectations are aligned.

At this point you will also potentially engage with fish brokers who are always looking for product. Cover yourself with a well thought our Supplier Agreement. We will be explaining what is critical in these types of agreements, particularly if you are entering the export market, for your business.

The logistics of getting product to market on time and within standards also comes into play. In this scenario it is important to understand the delineation of responsibility for the product as it moves from the farm to the fish market and on to the customer.

Growth can also mean new relationships. You may want to partner, move into a joint venture or sell your business. A business and legal professional can not only work up the agreements you will need but they can be instrumental in monetizing your business and working with your Accountant.

Advances in Farm Techniques

Aquaculture is one of the fastest growing food production industries. However, there are issues that can be managed with the addition of technologies. These technologies require special consideration in agreements to address issues such as using the product to standards, warranties and guarantees, the product is ‘fit for purpose’ and works in your ponds, to name a few.

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New Technologies

This paper will also spend some time on how robotics, blockchain and AI can assist in commercialising your business. While these technologies are fantastic for not only managing your ponds, blockchain means that you can implement smart contracts to assist with the majority of your external relationships that get product to market.

It will explore when a farmer work in experiments and trails with product and technology providers.

Outcomes from this Presentation
Understand the legal framework that your aquaculture business operates within
Gain insight into relevant legislations to protect your assets and intellectual property
Become experienced at knowing when to put Terms and Conditions into your business
Prevent disruption to your business through legal disputes.

OPPORTUNITIES AND CHALLENGES FOR UPSCALED GLOBAL BIVALVE SEAFOOD PRODUCTION

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Summary

Slow growth in the bivalve mariculture sector results from production inefficiencies, food safety concerns, limited availability of convenience products and low consumer demand. Here we assess whether bivalves could meet mass-market seafood demand across the bivalve value chain. We explore how bivalve production could become more efficient, strategies for increasing edible meat yield and how food safety could be improved through food processing technologies and new depuration innovations. Finally, we examine barriers to consumer uptake, such as food allergen prevalence and bivalve preparation challenges, high- lighting that appealing and convenient bivalve food products could provide consumers with nutritious and sustainable seafood options—and contribute positively to global food systems.

This abstract is based upon our recent publication in *Nature Food*, alongside unpublished data from research we have been carrying out with leading academics and global food companies. Please see Willer, D. F., Nicholls, R. J. & Aldridge, D. C. Opportunities and challenges for upscaled global bivalve seafood production. Nat Food 2, 935–943 (2021).

Benefits of unfed bivalve mariculture

To assess the sustainability and nutritional merits of bivalves relative to a current mass-market seafood, we compare bivalves with cod and Alaska pollock, alongside tuna (yellowfin and skip- jack). These wild-caught fish are already a proven resource and will continue to be a key component of consumer seafood, but supply is limited and global food companies are interested in how alternatives for filling the demand gap, such as bivalves, directly compare. In depth sustainability and nutritional analyses will be presented in the oral presentation alongside visual illustration.

Production

We discuss three key challenges to upscaling bivalve production: providing adequate volumes, maintaining sustainability, and ensuring food safety. We outline the emerging opportunities to tackle these challenges. These include new bivalve breeding approaches, feed technologies and grow-out practices; shell valorisation, the use of new species and sustainability regulations; and new depuration approaches including antimicrobial peptides, bacteriophages and chelating agents. We present several figures, including Figure 1, which outline the potential for production increases.

Food Processing

Bringing bivalve-based foods to the mass market will require creating bivalve products that can be easily and safely stored, distributed and purchased by consumers. We discuss the key challenges and opportunities in producing the major types of bivalve food products: fresh in-shell products, frozen de-shelled products, and cooked processed products. These are briefly outlined in Figure 2.

Consumer Uptake

We consider the key barriers to driving increased consumption of bivalves by the mass market and discuss the key opportunities to meet these challenges. We present the results of a consumer survey carried out with Europe's largest frozen food company (Nomad Foods) which can help us understand how to increase mass market demand. These results are compared with other global studies.

Conclusion

Demand for seafood is growing at double the rate of the global population, requiring nutritious seafood to be delivered to this mass market while avoiding damage to ecosystems through overfishing or poorly managed aquaculture. We discuss how through new innovation across production, food processing, and consumer uptake, bivalve shellfish can help meet this consumer demand.

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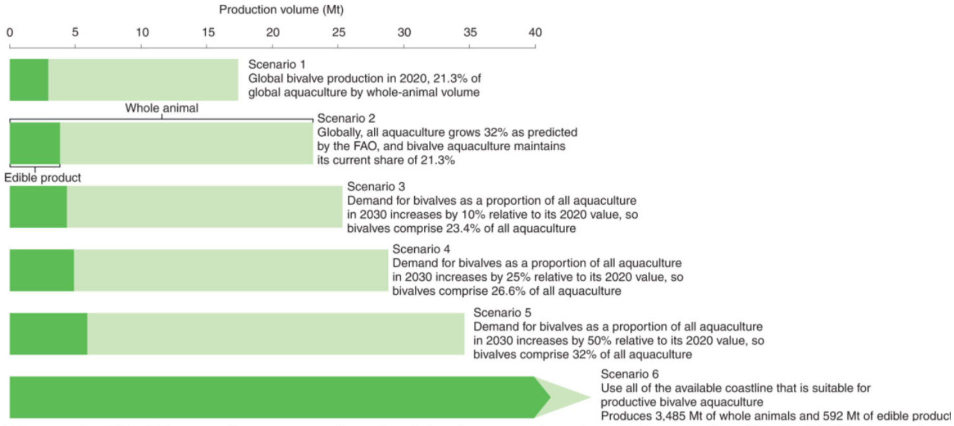
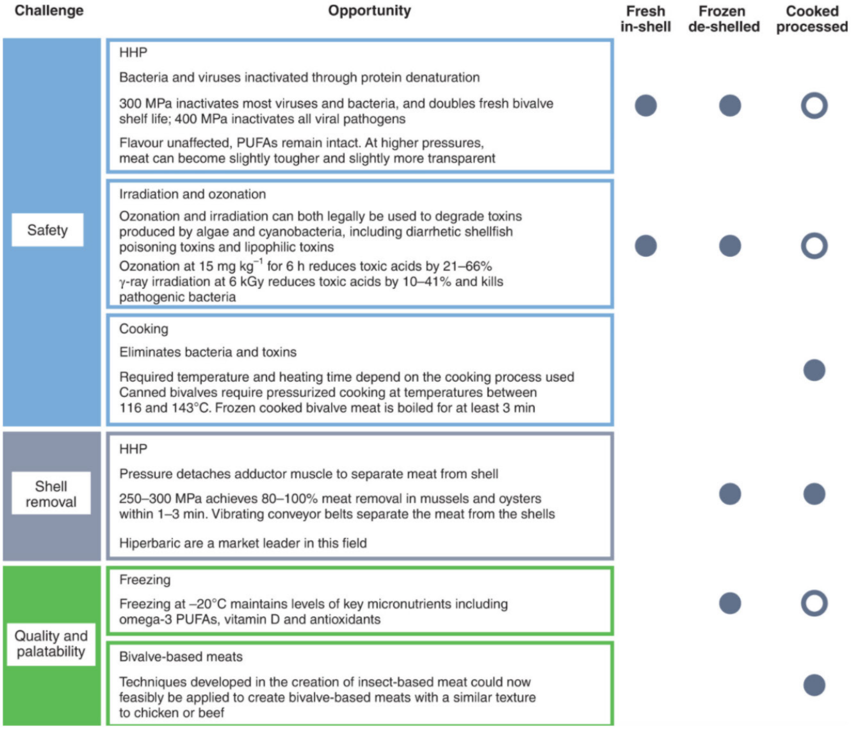


Figure 1. Six illustrative scenarios for bivalve production. Figure details in Willer et al. *Nature Food* 2021.



Challenge	Opportunity	Fresh in-shell	Frozen de-shelled	Cooked processed
Safety	HHP Bacteria and viruses inactivated through protein denaturation 300 MPa inactivates most viruses and bacteria, and doubles fresh bivalve shelf life; 400 MPa inactivates all viral pathogens Flavour unaffected, PUFAs remain intact. At higher pressures, meat can become slightly tougher and slightly more transparent	●	●	○
	Irradiation and ozonation Ozonation and irradiation can both legally be used to degrade toxins produced by algae and cyanobacteria, including diarrhetic shellfish poisoning toxins and lipophilic toxins Ozonation at 15 mg kg^{-1} for 6 h reduces toxic acids by 21–66% γ-ray irradiation at 6 kGy reduces toxic acids by 10–41% and kills pathogenic bacteria	●	●	○
	Cooking Eliminates bacteria and toxins Required temperature and heating time depend on the cooking process used Canned bivalves require pressurized cooking at temperatures between 116 and 143°C. Frozen cooked bivalve meat is boiled for at least 3 min			●
Shell removal	HHP Pressure detaches adductor muscle to separate meat from shell 250–300 MPa achieves 80–100% meat removal in mussels and oysters within 1–3 min. Vibrating conveyor belts separate the meat from the shells Hiperbaric are a market leader in this field		●	●
Quality and palatability	Freezing Freezing at −20°C maintains levels of key micronutrients including omega-3 PUFAs, vitamin D and antioxidants		●	○
	Bivalve-based meats Techniques developed in the creation of insect-based meat could now feasibly be applied to create bivalve-based meats with a similar texture to chicken or beef			●

Figure 2. Key opportunities for producing fresh in-shell, frozen de-shelled and cooked processed bivalve products. Figure details in Willer et al. *Nature Food* 2021.

ENZYME ACTIVITY AND PLASMA NUTRIENT VARIATION DEPENDENT ON FEEDING TIME IN ATLANTIC WOLFFISH (*Anarhichas lupus*)

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Introduction

Atlantic wolffish (*Anarhichas lupus*) has been proposed as a cold-water species in aquaculture [1]. However, in order to explore the commercial farming potential of the species, we need more knowledge about its nutritional and digestive physiology, nutrient requirements and feeding protocols for optimal growth and welfare. Previous studies in fish on protein digestion, including a few on Atlantic wolfish, show that the enzymatic activity of intestinal trypsin and chymotrypsin is well correlated with growth, and feed conversion efficiency [2–4]and of 6°C induced the variant TRP-2*92 ($p<0.01$. Digestion leads to nutrient uptake and increased plasma concentrations of amino acids, glucose and lipoproteins. Plasma amino acid and glucose concentrations of fed Atlantic wolffish has not been measured. In other fish species dependent on feeding strategy and intestinal morphology, digestion of protein and amino acid uptake result in increased free amino acid plasma concentration short after feeding [5]. Further, lipase activity and fat absorption have not been examined in Atlantic wolffish. Free fatty acids (FFA) plasma concentration is known to vary between species and have seasonal and circadian variations [6,7].

The aim of the current study was to gain knowledge about the diurnal and nocturnal activity of digestion of protein and lipids in Atlantic wolffish.

Material and method

Atlantic wolffish juveniles were kept in re-circulating water systems with artificial seawater at 10˚C, and artificial 12:12-h light-dark photoperiod. The fish was fed in excess to visual satisfaction once a day for 4 weeks in the morning (MF) (8 am), or evening (EF) (8 pm) (Skretting Amber Neptune grade 2.0, Skretting, Stavanger, Norway). The fish were fasted 24 h before sampling. On the sampling day, the fish were fed once and after 30 min, uneaten feed was collected. Sampling of chyme, intestinal scrapings and plasma started 1h post feeding with subsequent sampling after 7, 13, 19 and 25 h for each feeding regime. Intestinal enzymatic activity of trypsin, chymotrypsin and lipase as well as plasma profiles of free amino acids (FAA), free fatty acids (FFA) and glucose were measured.

Results and conclusion

In both feeding groups, there was no difference in trypsin and chymotrypsin activity in the proximal mid-intestine over time (Fig 1 A and B), whereas there was higher of chymotrypsin activity in the distal mid-intestine in MF fish 1 h post feeding (not shown), and lipase activity remained un-changed during the whole experimental period (not shown). In MF fish, there was a decrease in trypsin and chymotrypsin activity in the proximal mid-intestine after 19 h that was correlated with a drop in hepatic total FAA concentration and glucose, suggesting that the fish goes into a digestive resting state during the dark cycle (Fig. 1 C and D). This effect was not seen in EF fish that had sustained enzyme activity and high plasma FAA, and glucose concentrations through the experimental period.

In conclusion, Atlantic wolffish has a higher and more stable trypsin and chymotrypsin activity along with increased plasma FAA and glucose plasma concentration if they are fed in the evening compared to if they are fed in the morning. Since feeding time influence the digestive ability and nutrient uptake over time after an ingested meal this information could be used to optimize the feeding strategy for Atlantic wolffish. Future studies should aim for examining the long-term growth effect under different feeding regimes and continuous feeding.

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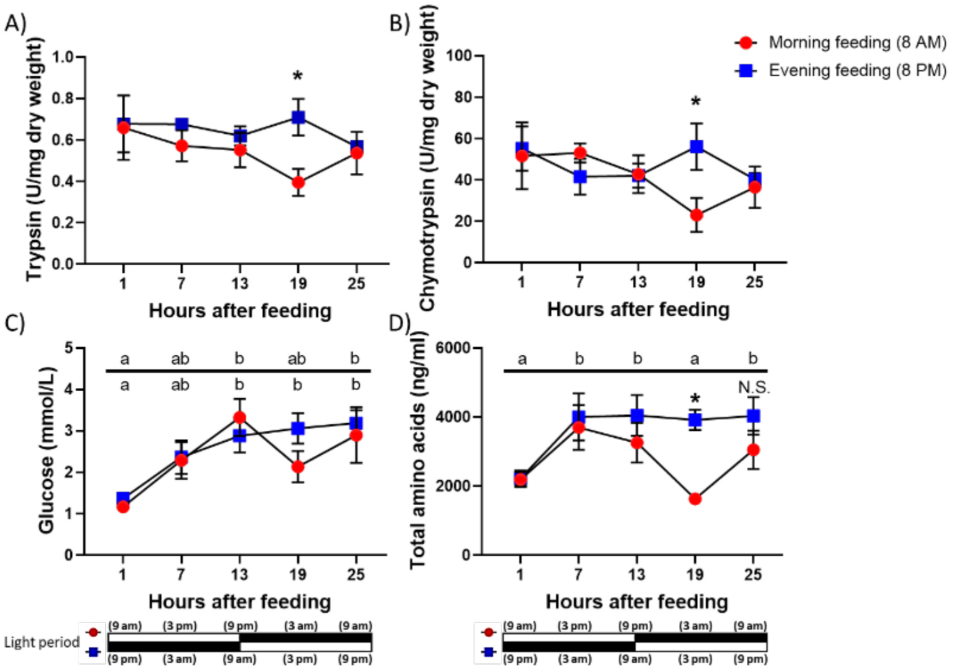

*Figure 1. Intestinal enzymatic activity of A) Trypsin and B) chymotrypsin in the proximal mid-intestine and nutrient plasma concentration of C) glucose and, D) total free amino acids after 25 h post feeding in juvenile Atlantic wolffish. Significant difference between morning and evening fed fish is indicated by *. Significant difference over time is indicated by different letters.*

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IMPROVING MICRODIET FORMULATION FOR TWO FLATFISH SPECIES: SENEGALESE SOLE AND ATLANTIC HALIBUT

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Introduction

Flatfish species such as Senegalese sole and Atlantic halibut are target species for European aquaculture diversification due to its high market value and excellent consumer acceptance. Aquaculture development of these species partially relies on optimization of feeding and nutrition, especially at early development stages. Although significant progress has been made on microdiet quality, feeding regimes and zootechnical procedures for Senegalese sole (Pinto et al., 2018), little R&D work has been conducted to improve microdiets for Atlantic Halibut larvae and post-larvae. Atlantic halibut poses difficulties on accepting inert microdiets (Hamre et al., 2019) which can lead to increased mortalities and depressed growth rates. Therefore it becomes paramount to carefully formulate and produce microdiets with suitable nutritional and physical properties, as well as increased attractiveness targeting the early developmental stages. This work presents two trials which were conducted to evaluate the effect of several microdiets with varying protein sources on the growth performance and survival of Senegalese sole and Atlantic halibut.

Material and methods

The trial with Senegalese sole aimed at identifying the preference for a specific protein source and comprised three dietary treatments: MIX (mixture of marine and plant-based ingredients), KRILL (krill meal) and SQUID (squid meal). Microdiets were introduced in the feeding regime of Senegalese sole postlarvae at 27 days after hatching (DAH) and kept for nearly 6 weeks (68 DAH). Sole postlarvae were reared at an initial density of 3000 fish/m^2 in triplicate flat-bottom tanks (8 L), set in a partially-closed recirculating aquaculture system. Sole were sampled at 27 DAH, 51 DAH and 68 DAH. The second trial was performed also to determine Atlantic halibut's preference for a main protein source. Three dietary treatments were used: MIX, SQUID and KRILL. Atlantic halibut postlarvae were reared for 35 days, from 97-132 DAH and were kept in triplicate flat-bottom tanks (2x2m). Sampling occurred at 97, 115 and 132 DAH. Both trials comprised the analyses of Relative Growth Rate (RGR), Feed Conversion Ratio (FCR) and Survival. Eye migration, pigmentation quality and liver histology were also evaluated in the Atlantic halibut trial.

Results

At the end of the sole trial, no differences were found on dry weight amongst the 3 treatments (Figure 1). In addition, there were no differences in RGR (9-9.4%), FCR (0.96-1.08) and survival (57-66%). On the other hand, Atlantic halibut group fed on the Squid diet showed significantly lower wet weight (figure 1) and also the lowest total length ($P<0.05$) at the end of the trial. There were no significant differences in RGR (3.02-3.77), FCR (2.3-3.3) and survival (>77%).

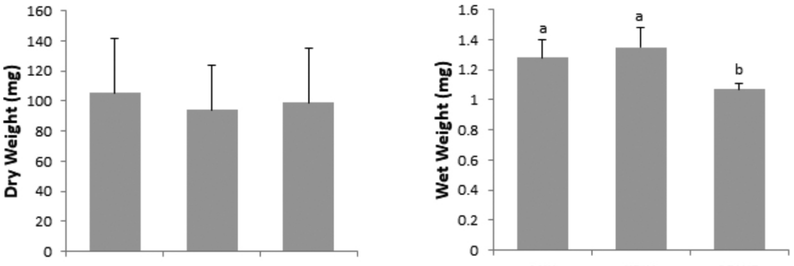

Figure 1. Dry weight of Senegalese sole (left) and Wet Weight of Atlantic halibut (right) fed diets with different protein sources – Mix (mixture of marine and plant-based ingredients), Krill (krill meal) and Squid (squid meal). Results are expressed as means ± standard deviation. Different lowercase letters indicate significant differences between the dietary treatments ($P<0.05$).

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Discussion and Conclusion

There is scope to keep testing novel feed formulations which can promote growth at early development stages of emergent aquaculture fish species such as Senegalese sole and Atlantic halibut. The present results show that sole performed well on all tested microdiets, regardless the protein source. The Relative Growth Rate (RGR, up to 9.46 % day^{-1}) is satisfactory although slightly behind the 12-13 % day^{-1} achieved nowadays during weaning. Such high RGRs are only possible due to the intensification of R&D efforts on microdiet optimization for Senegalese sole in the last decade (Pinto et al., 2018). On the other hand, Halibut seem to have a preference for diets with a mixture of marine and plant-based ingredients (MIX) or krill meal (KRILL). Both diets resulted in improved growth performance when compared with the diet with squid meal (SQUID). Interestingly, both species have different feeding behaviours; whilst sole is a typical passive bottom feeder, Atlantic halibut shows a high swimming activity especially after metamorphosis (Kristiansen et al., 2004), being a more proactive feeder. Overall the results obtained on microdiet preference for both species and also the distinct feeding behaviours suggest that diet formulation and physical properties should be tailored to each species in order to improve attractiveness and maximize growth performance.

Acknowledgements

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CHROMOGRANIN A AS STRESS MARKER IN FISH

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Introduction

Chromogranins belong to the granin family, soluble acid proteins which are expressing in several endocrine, neuroendocrine and neuronal cells. They are stored in secretory granules and released together peptidic hormones, neurotransmitters or amines as response to a range of stimuli (D'amico et al., 2014). The chromogranin A (CgA) has recently reported as stress marker in superior vertebrates (Srithunyarat et al., 2018). Its higher stability in blood than catecholamines, not being submitted to circadian rhythms and easier analysis and preservation make them a remarkable candidate for stress markers based on the SAM (sympathetic-adreno-medullar) axis reactivity (Martínez-Miró et al., 2016).

Although the proprieties of peptides derived from chromogranins acting as cardiac stabilizers have been studied in fish, their relationship to the stress response has been not described in any work yet (Imbrogno et al., 2019). In this sense, it has been reported that these peptides show cardio-inhibitors effects in teleosts and use several strategies for the cardiac control, despite its biological meaning still remains unclear.

The objective of this work was to study the chromogranin A variation for stress in fish, aiming at determining if those proteins could be a suitable stress marker, mainly for chronic stress conditions.

Material and methods

The experimental cultures were carried on 900 L circular tanks (0.65 m radius) integrated in a close water system. Meagre juveniles were purchased from MARESA (Ayamonte, Spain) and submitted to an acclimation period for two weeks. After fish were initially sampled for body weight (181.5±15.1 g) and total length (37.3±0.88 cm).
Chronic stress culture: It consisted of two treatments, stressed (CS) and control (CT) for 6 months. The CS groups were submitted to confinement and netting/chasing stress.

Acute stress challenge: After completing the chronic stress culture, all remaining fish from the control groups were kept in the same non-stressing conditions for15 days. Then 10 fish were netted and exposed to air stress for 3 minutes, and sampled after 15 minutes.

Fish were sampled at the end of the experimental chronic stress culture (6 months) and the air stress challenge for blood, tissues and biometry. Plasma glucose, proteins, lactate and cortisol were determined, as well as brain and head kidney cortisol and chromogranin A.

Results and discussion

Acute stress

Plasma lactate and cortisol increased significantly after stress although glucose and proteins remained stable (Fig. 1A, 1B). As plasma cortisol, kidney cortisol after stress was significantly higher (Fig. 1B). Kidney CgA decreased significantly in the stressed fish though brain CgA did not change between statuses (Fig. 1C).

Chronic stress

Several biometric parameters varied between treatments significantly (Table 1). They were significantly lower in chronically stressed fish. Plasma stress markers did not change significantly (Fig. 2A, 2B). Kidney cortisol increased in stressed fish (Fig. 2B). Both brain and kidney CgA concentrations decreased in stressed fish (Fig. 2C).

Only kidney CgA and cortisol kept the same variation pattern in both stress types. Although cortisol concentrations in plasma and tissues have been widely studied, the tissue CgA concentrations related to stress have not still reported in fish. Initially, the depletion of kidney CgA could be considered as a chronic stress marker.

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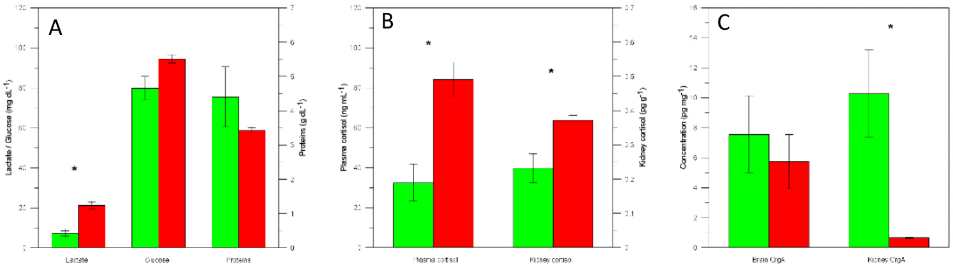

Fig. 1. Plasma glucose, lactate and protein (A), plasma and kidney cortisol (B), and brain and kidney CgA (C) measured for each treatment. Green and red bars are for non-stressed and acute stressed groups, respectively. Asterisks indicate significant differences ($p<0.05$) between groups.

Table 1. Biometric parameters registered for non-stress and chronic stress treatments. Asterisks indicate significant differences between non-stressed and stressed status.

	Non-stressed	Chronically stressed
Initial weight (cm)	181.5±15.1	
Initial length (cm)	27.2±0.88	
Final weight (g)	403.1±29.1 *	302.3±46.8
Final length (g)	34.5±0.93 *	31.5±1.36
SGR (day^{-1})	0.41±0.04 *	0.22±0.08
K	0.94±0.02 *	0.90±0.02
Survival rate (%)	75±5	68±2.5

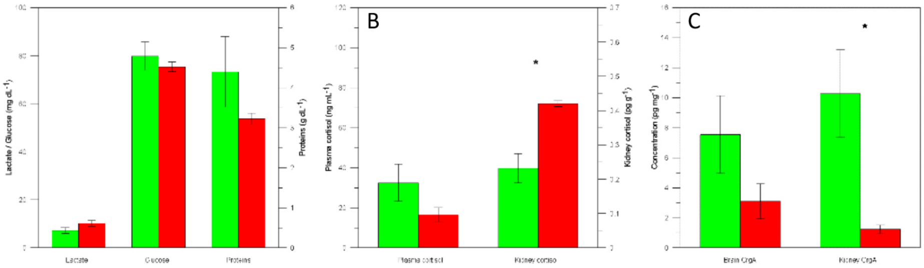

Fig. 1. Plasma glucose, lactate and protein (A), plasma and kidney cortisol (B), and brain and kidney CgA (C) measured for each treatment. Green and red bars are for non-stressed and chronically stressed groups, respectively. Asterisks indicate significant differences ($p<0.05$) between groups.

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PROSPECTIVE LONGITUDINAL STUDY OF PUTATIVE AGENTS INVOLVED IN COMPLEX GILL DISORDER IN ATLANTIC SALMON

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Introduction

Gill diseases are an important challenge for Atlantic salmon (*Salmo salar*) aquaculture worldwide. Complex gill disorder (CGD) is a multifactorial and multiaetiological condition that is thought to be a consequence of the interaction of a number of factors including environment, management practices and pathogenic microorganisms. The main infectious agents associated with cases of CGD in Atlantic salmon are *Candidatus* Branchiomonas cysticola, *Desmozoon lepeophtherii* and salmon gill poxvirus (SGPV) (Herrero et al. 2018)pathogenic organisms and farm management practices. None of the three principal agents purportedly associated with CGD (Desmozoon lepeophtherii, salmon gill poxvirus or Candidatus Branchiomonas cysticola. The individual roles and possible interactions between these pathogens have not yet been fully elucidated and studies on infectious gill disease agents in Scottish aquaculture, other than *Neoparamoeba perurans*, are scarce. In the absence of *in vivo* or *in vitro* experimental models, prospective longitudinal studies can help to clarify any associations between exposure to a potential aetiology and the development of disease.

Material and methods

A prospective longitudinal study was designed to investigate the infectious dynamics of the putative pathogens of CGD for one year, starting from the late freshwater stage of the production cycle and continuing through the subsequent marine stage. The protozoan *N. perurans*, which causes a specific disease known as amoebic gill disease (AGD), was also included in the monitoring of the study. The relative loads of the four pathogens, estimated using real-time reverse transcriptase polymerase chain reaction (RT-rtPCR) Ct values, were correlated with a semi-quantitative histological gill scoring system derived from the samples. Generalised additive models (GAMs) were used to represent changes over time in the Ct values of the different infectious agents in the gills of salmon at the various sampling timepoints in the two farms, and to represent the variation of the gill score across time and farms. Linear regression models were used to study the possible associations between gill score and different explanatory parameters such as variations in the pathogens' loads, environmental and farm management factors.

Results and discussion

The two marine farms screened in this study were positive for the three main pathogens associated with CGD by RT-rtPCR, which shows that the detection of *D. lepeophtherii*, *Ca.* B. cysticola, and SGPV are common in marine Scottish salmon farms. *Ca.* B. cysticola and *D. lepeophtherii* were the most prevalent agents, whilst SGPV was detected irregularly throughout the study. In addition, *Ca.* B. cysticola and SGPV were first detected in the late freshwater stages of both populations suggesting that these pathogens were carried from the freshwater to the marine farm.

In the marine phase, Farm B showed significantly higher gill scores compared to Farm A ($p< 0.05$). Farm A experienced minimal to mild unspecific gill changes during the study whilst fish in Farm B had gill pathology characterised by AGD lesions and low to occasionally moderate vascular, proliferative and inflammatory pathology, from late summer until early winter. Gill score was significantly higher during the autumn on both farms compared to other seasons ($p< 0.05$). The strong link between the gill score and seasonality may indicate that water temperature is an important risk factor in terms of gill health compromise. In this study, the load variation of SGPV was not significantly associated with changes in the gill score ($p> 0.05$) and typical pathology associated with SGPV was not detected. As fish seemed to be first infected during the freshwater stage, it could be that the virus becomes latent and can be re-activated at a later stage, for example during episodes of immunosuppression, contributing to the gill pathology. An increase in *Ca.* B. cysticola load was associated with an increase in the gill score ($p< 0.05$) when only Farm B was used in the statistical analyses. *Ca.* B. cysticola was shown to be associated with gill pathology by *in situ* hybridization, for CGD events occurring in the absence of visible epitheliocysts, showing that the effects of the bacteria can be easily overlooked by common histological methods (Gjessing et al., 2021).

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In this study, the inflammatory and necrotic cell reaction in the gills was not marked (changes associated with *Ca*. B. cysticola), and the low level of epitheliocysts detected in the histopathology suggests the bacterium was not a major cause of the gill disease present in Farm B although it could have contributed to some of the changes present. Higher loads of *D. lepeophtherii* were significantly associated with an increased gill score in Farm B ($p < 0.05$) but not in Farm A ($p > 0.05$), and characteristic *D. lepeophtherii*-type microvesicles were minimal and not significant in the gills of fish examined from both farms, which suggests the significant associations could be the result of the parasite developing in more affected gills (with higher gill score) rather than *D. lepeophtherii* being a causative agent of the gill pathology observed. An increase in *Neoparamoeba perurans* loads was significantly associated with an increase in gill score ($p < 0.05$) on both farms and an outbreak of amoebic gill disease was one of the main causes of the gill disease observed in Farm B.

When using only Farm B in the linear model, parameters such as a lower number of days since last net cleaning and a lower number of days since the last hydrogen peroxide treatment were significantly associated with an increase in the gill score ($p < 0.05$). *In situ* net-pen pressure washing cleaning of the nets and the mechanical trauma caused during the treatments performed likely contributed to the gill damage observed in Farm B (Østevik et al., 2021, 2022).

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MARINE INGREDIENTS FOR FEED FOR AQUACULTURE: AN ANALYSIS OF CERTIFICATION AND SUSTAINABLE SOURCING

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Introduction

Global fish production peaked at about 171 million tonnes in 2016, with aquaculture representing 47 percent of the total and 53 percent if non-food uses (including reduction to fishmeal and fish oil) are excluded (FAO, 2020). Global aquaculture production (including aquatic plants) in 2016 was 110.2 million tonnes, with the first-sale value estimated at USD 243.5 billion (FAO, 2018). The total production included 80.0 million tonnes of food fish, 30.1 million tonnes of aquatic plants as well as 37,900 tonnes of non-food products (USD 214.6 million). EU aquaculture accounts for about 20% of fish and shellfish supply in the EU and directly employs about 70,000 persons. EU aquaculture accounts for about 20% of fish and shellfish supply in the EU. Despite COVID-19 complications like high level of absenteeism at work sites, transportation difficulties, limited capacity for diagnostic tests for COVID-19, and an increase in operating and logistics costs mainly, global production has been increasing between 5% to 7%.

Despite the diversity of aquaculture, the EU aquaculture production is largely concentrated on a few species, the most important being mussels, salmon, seabream, rainbow trout, seabass, oysters, and carp. Looking at the diversity of species but also the farming method, we see a high dependency on feed for salmon, seabream, rainbow trout, seabass, and carp.

On the other hand, Industry more and more has been attacked by the media specifically because of the environmental and sustainable impact that this is producing. At the same time, there is a very close relationship and synergy with fisheries because it is a fact: The aquaculture industry will not exist without feed and marine ingredients mainly. Marine ingredients include essential proteins, fats, vitamins, and minerals that are important for farmed fish, and are also passed on to humans when they eat farmed seafood. Growing global production from aquaculture, as envisioned by the United Nations' Food and Agriculture Organization in its recent "State of World Fisheries and Aquaculture 2020" report, will require additional feed and a greater demand for marine ingredients use of marine ingredients in aquafeed will not decline soon,

Feed companies are becoming more sophisticated in using the nutritional and physical properties of materials, but the growth in marine ingredients derived from byproducts still needs more focus, currently, more than one-third comes from the byproducts of wild or farmed fish processing, but there is potential for greater innovation. Feed manufacturers already regard them less as a commodity and more as a strategic ingredient in feed made from a wider variety of feed components. According to the FAO forecast, by 2050, it will be necessary to triple protein production to support the development of 10% annual aquaculture production. Arable land is limited and stocks of fish are strained. It is necessary to find additional resources rich in protein in a market of 9 billion euros. Alternative ingredients with similar nutritional value profile, and from marine origin should be a solution, and if it is possible to produce it in a sustainable manner

Methods and Materials

A key part of our methodology is to compile and review current aquaculture certification programs and schemes certifying marine ingredients and feed for aquaculture. How they are addressing the marine ingredients for feed in the supply value chain and the interdependency among aquaculture and fisheries certification programs. A full reference list will be shared during the presentation of this paper.

The scope of the paper and gap analysis include aquaculture and fisheries certification programs associated with shrimp production. A case study of different alternative marine ingredients has been reviewed and valuated. Therefore we have tried to examine these sequentially and have attempted to produce an assessment list for considering at the moment of assessing a new feed marine ingredient and/or alternative – decision making. Whilst we have provided an overview of the marine ingredients for feed in aquaculture and alternatives sources, we have not examined this in detail as several global feed producers may have done in the private sector.

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Results and Conclusions

A stairs process approach will be shared as well as reflections in the European feed marine ingredients and 8 steps to consider for feed ingredients design. The right certification scheme for your ingredients and feed seems to end in only one certification program – Marin Trust. Reflections of the important requirements are part of the conclusions.

Beyond certifications, the feed producers should start to develop an assurance model that includes KDEs for a continuous evaluation of the feed ingredient composition, origin, nutritional value, food safety, quality, sustainability, and labor conditions of workers in the supply chain.

WIND-WIN: A MULTI-USE CONCEPT FOR INTEGRATING NON-FED AQUACULTURE WITH OFFSHORE WIND ENERGY GENERATION

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Introduction

Off-shore marine environments are often portrayed as new frontiers for development by international organizations such as the EU and the UN (European Commission, 2017, FAO, 2017). The expansion of marine exploitation is set to reduce stresses on limited terrestrial environments through the sustainable harnessing of untapped resources at sea, to use technological advancements and to maximize social benefits whilst minimizing social resistance (WorldBank, 2017). While new technology makes these resources more accessible than ever, stakeholder engagement and regulatory expediency are critical to realizing the full potential of development in this arena. A framework providing accessibility for stakeholders as well as predictability for investors has the potential to accelerate development. To increase transparency and to reduce the areas of conflict, Marine Spatial Planning (MSP) has become a commonly used tool to plan and consider different claims and stakeholder positions (Qiu and Jones, 2013). MSP has also become a staple in Blue Growth initiatives that are aimed at enabling and nudging states towards expanding marine development. Presented here is a scalable framework for successful implementation of coupled offshore aquaculture and renewable energy, as well as a case study in the Baltic Sea, one of the most eutrophicated environments in the world.

Case study: macroalgae cultivation in the Baltic Sea

In Europe, the potential for both micro- and macroalgae cultivation is slowly being recognized and encouraged as a powerful tool in the face of urgent challenges, including climate change, food and energy security and social prosperity. Although the number of seaweed farmers is increasing, the majority of the biomass still comes from foraged wild seaweed. With the growing market for bio-based products and the increased knowledge of how to utilize seaweed, there is huge potential throughout the whole supply chain for creating an industrial-scaled macroalgae industry in Europe. The potential global seaweed market value is estimated at € 9.3bn by 2030, creating some 115 000 jobs while improving the environmental state of our oceans (Vincent et al., 2020).

With its low temperatures and brackish waters, the Baltic Sea is home to a unique set-up of marine species, including some 450 macroalgae species. The low salinity level hinders the cultivation of the more conventional macroalgae species, nonetheless, there are a number of species that are of interest, both based on their properties as well as from an economic perspective. Furthermore, seaweed is considered a promising protein for food, feed and the bio-based economy (van den Burg et al., 2020). These include, but are not limited to, the brown algae *Fucus vesiculosus* (bladderwrack), red algae *Furcellaria lumbricalis* and green algae *Ulva lactuca* (Sea lettuce) and Ulva intestinalis. At the present time, there are small-scale pilot projects studying the potential of macroalgae cultivation in the Baltic Sea whereas wild seaweed is commercially foraged only in Estonia and Denmark.

Implementation framework

The Wind-Win project framework is a tool to create and identify a network of actors benefiting from the multi-use concept both on a local and international level. The concept combines identification of local partners, expert technical analysis of aquaculture production elements, as well as developing infrastructure both onshore and offshore including job opportunities and business incentives. Special emphasis is placed on developing the necessary associated onshore hatcheries and nurseries in synergy with local industries based on the circular economy model. Introducing a multi-use approach of ocean space, combining aquaculture and offshore wind, provides a unique opportunity to leverage local tourism to bring awareness about both sectors.

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Methodology

An array of methods from diverse disciplines are employed to assess the viability of offshore multi-use development. As achieving stakeholder engagement is a major challenge for projects of this scale, significant attention was given to developing an adaptable outreach strategy including branding approaches and local market development. Technical requirements for operations including tradeoffs between single and multi-use facilities are provided alongside conceptual designs and review of technological developments contributing to feasibility of projects within the framework. Furthermore, a Strengths, Weaknesses, Opportunities and Threats (SWOT) analysis is included as a means to preemptively identify specific elements likely to influence the outcome of the project.

Conclusion

New legislation and heightened incentives have generated momentum towards a transition to green energy in Europe (European Commission, 2021c). The war in Ukraine has only stressed this need further and many states are pushing for a quicker transition to clean energy, more specifically wind (Tollefson, 2022). The development framework proposed here aligns with the Green New Deal and Blue Growth initiatives that emphasize energy, especially through off-shore wind farms to expedite growth in this sector as well as facilitate the de-eutrophication of European Waters (European Commission, 2019, 2022). As a resilient, low impact addition, seaweed farming is a perfect candidate for multi-use in a variety of habitats (Wever et al., 2015, Bak et al., 2018). The planned expansion of windfarms in offshore environments is driving a multi-use interest. The Wind-Win framework and associated Baltic Sea case study provide the structure for rapid implementation of this promising partnership between green energy and blue growth.

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MINERAL CARRY OVER FROM PLANT-BASED AND MARINE-BASED FEEDS ON SPOTTED WOLFFISH (ANARHICAS MINOR): EFFECTS ON GROWTH, HEALTH AND WELFARE

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Introduction

The spotted wolffish, *Anarhichas minor,* is a promising species for coldwater aquaculture (Foss *et al.,* 2004; Le Francois *et al.,* 2021). The nutritional requirements may not be fully met in captivity, which can result in health issues such as tooth loss and neprocalcinosis (Foss *et al.,* 2004; Béland *et al.,* 2020). Previous studies have shown the potential of marine and plant based alternative protein sources in wolffish diets, with no negative effects on growth and stress markers such as plasma cortisol, glucose and lactate (Knutsen *et al.,* 2019[a,b]). Whereas, other more recent investigations imply no significant effects on intestinal barrier function and nutrient uptake by alternative marine protein sources (Hinchcliffe *et al.,* in prep). However, health issues like kidney stones and tooth loss remain (Béland *et al.,* 2020; Le Francois *et al.,* 2021). Little attention has focused on mussel meal as a potentially beneficial ingredient in aquafeeds for wolffish. Blue mussels have protein and amino acid content similar to fishmeal and can act as both a dietary protein source (Vidakovic et al., 2016; Langeland et al., 2016) and feed attractant for fish (Berge and Austreng, 1989). Moreover, the shell of mussels (BMS) can act as a source of minerals such as calcium and phosphorous, that are essential for health (Flik and Verbost 1993). Its potential benefit for maintaining bone density and reducing kidney stone levels is yet to be investigated for any fish species. The aim of the current study was to investigate the inclusion of mussel meal and shell in marine and plant based on-growth diets for spotted wolffish in a 30 week feeding trial. With specific objectives to:

1. Establish effect of plant based ± BMS *vs.* marine based diets ± BMS on growth performance and plasma health and welfare markers of spotted wolffish
2. Establish effect of experimental diets on gut barrier function and nutrient transport using Ussing chamber technology
3. Compare neprocalcinosis and bone density in diets using CT scans.

Material and methods

For the study, 600 fish 104g ± 15.18 (mean weight ± SD) were randomly distributed into 12 PE-coated fiberglass tanks (1200 L), triplicate tanks (50 fish/tank) were allotted to a treatment, and fed one of 4 experimental diets. 1) **MC**: 60% fishmeal, 20% blue mussel meal. 2) **MS:** 61.7% fishmeal, 20% blue mussel meal with shells. 3) **PC**: 30% fishmeal, 20% pea and soy protein concentrates and 20% blue mussel meal. 4) **PS:** 30.9% fishmeal, 19.5% pea and soy protein concentrates and 20% blue mussel meal with shells. At the start, middle and end of the feeding trial, weights and lengths of all individual fish were recorded. At the end of the experiment, liver weights for hepatosomatic index were measured and blood was sampled for plasma growth and appetite hormones, IGF-1 and ghrelin; stress parameters such as cortisol, glucose, lactate, ion content and acid-base balance and nutrient parameters such as FFA (n=12 fish/group). Ussing chambers were utilized to characterize gut barrier function and nutrient uptake in fish fed the different diets (n= 12 /group). Finally, we collected whole body of fish for chemical composition, tooth and bone structure and kidney stone quantification (n = 12 fish/group).

Results and discussion

At the end of the trial, fish showed a final weight that was approximately 5 times higher than their initial weights (Table 1). Overall, fish fed the plant-based diet with mussel meal (PC) and shell (PS) were the strongest performers; the final SGR of the fish fed these diets were significantly higher (0.65 ± 0.01 %/day) relative to fish fed the MC diet (0.60 ± 0.01 %/day). While the final weight of the group PC was significantly higher than the MC and MS treatments (522 ± 7.4 g *vs.* 462 ± 5.8 and 463 ± 8.4 g, respectively). In contrast, no significant differences were found for plasma IGF-1 which ranged between 12-14 ng/ml in all treatments. A significant increase in HSI was found in marine based diets relative to plant-based diets (4.53 ± 0.13 *vs.* 4.04 ± 0.13). Plasma FFA concentrations have been measured but pend further investigation to ascertain consequences on lipid metabolism from the marine based diets. In addition, other hormones related to appetite

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Table 1. Growth and health indices of the study groups.

Parameters	MC	MS	PC	PS	P value
Initial weight (g)	103.84 ± 0.77	103.26 ± 0.22	106.03 ± 1.73	102.92 ± 0.89	0.231
Mid-way weight (g)	228.54 ± 2.94	222.88 ± 2.97	243.03 ± 8.31	231.67 ± 6.57	0.163
Final weight (g)	462.14 ± 5.72^{a}	463.00 ± 8.40^{a}	521.83 ± 7.37^{b}	496.03 ± 13.42^{ab}	0.005
Mid-way SGR (%/day)	0.87 ± 0.01	0.85 ± 0.01	0.91 ± 0.03	0.89 ± 0.02	0.264
Final SGR (%/day)	0.60 ± 0.01^{a}	0.63 ± 0.02^{ab}	0.65 ± 0.01^{b}	0.65 ± 0.01^{b}	0.026
HSI (mid-way)	4.38 ± 0.16	4.29 ± 0.43	4.20 ± 0.12	4.28 ± 0.17	0.249
HSI (Final)	4.54 ± 0.14^{a}	4.52 ± 0.11^{a}	4.08 ± 0.13^{b}	3.99 ± 0.12^{b}	0.003

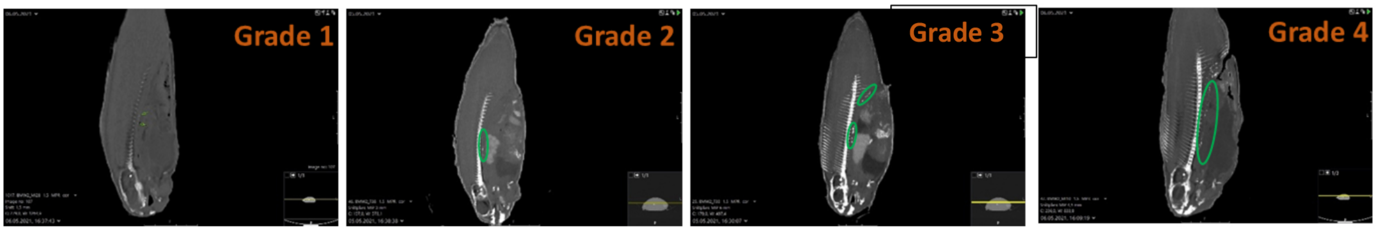

Figure 1. Kidney stone scoring system used in the present study

and stress/welfare, ghrelin and cortisol, are under current analysis and will be discussed further with data ascertained for ionic-balance and acid-base balance. Preliminary results from the neprocalcinosis scoring (Fig1) indicated no significant differences between the experimental diets, suggesting no clear evidence on the effect of diet on kidney stone formation. Bone density also did not differ significantly between the experimental diets. Kidney stone and bone density may well be correlated in spotted wolffish and this is pending further investigation that will be discussed along with the gut barrier function and nutrient uptake from the Ussing chamber methodology.

In conclusion, the present experiment shows the potential of plant-based protein sources, for the emerging spotted wolffish aquaculture sector with no significant impacts on growth or welfare observed. The preliminary data shows no clear evidence thus far on the effect of diets on kidney stone formation in spotted wolffish.

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ADVANCES IN EUROPEAN LOBSTER RESEARCH ON THE SWEDISH WEST COAST

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Introduction

The European lobster (*Homarus gammarus*) has been the subject of aquaculture research for the last 150 years, due to both the wild fishery and cultural value of the species. Since the middle of the 20th century, increasing anthropogenic pressure has led to a severe decline in wild fish and shellfish stocks, including *H. gammarus,* with some fisheries yet to recover. *H. gammarus* is amongst the most exclusive shellfish products worldwide, and whilst management measures have been implemented to safeguard stocks, *H. gammarus* aquaculture is an increasingly credible approach to help to secure sustainable lobster supply. Historically, this has been achieved by relatively small-scale release of hatchery reared juveniles. Until recently, commercial attempts at farming have not been viable, mainly due to cost prohibitive technical challenges. At present, there are several promising approaches to overcome bottlenecks hampering commercialisation of lobster aquaculture. This talk will summarise the new husbandry and nutritional advancements in *H. gammarus* culture from experiments performed at Swedens fiest pilot scale lobster hatchery over the last 5 years. Additionally, likely directions for both sectors in the coming decades are summarised, knowledge gaps identified, and the societal support required to achieve further potential will be highlighted.

Material and methods

This presentation will summarize data taken from a series of juvenile lobster exoeriments that took place between 2017-2022 at Kristineberg research station, Sweden. In general, gravid female lobsters were donated from commercial fishermen operating in Gullmarsfjorden, Sweden, and delivered directly to the Sven Lovén Centre, Kristineberg, Sweden during September- October 2015 and September-October 2016. During preparation for experimental periods, lobsters were individually maintained in opaque 40 L perforated boxes with a 8:16 L:D photoperiod, immersed in a flow-through system, incorporating “deep” water pumped to shore and originating from ca. 33 m depth. Temperature fluctuated with season but with a minimum and maximum 6-16°C. For hatching, up to three ripe adults were removed and placed in an experimental facility for preparation of the experiments. Hatching systems were matched to ambient water conditions, and temperature was gradually increased and temperature was gradually increased. For the experiment 1 larvae were fed a wet feed, dry feed and conspecific treatment in communal and individual reared cells to ascertain the degree and importance of larval cannibalism in lobster nutrition. In experiment II, Pandalus borealis, was used as a reference diet, with four additional diets: isocalorific and isonitrogenous commercial fishmeal, experimental shrimp meal, herring meal ± supplements, or mussel based feeds based on the nutritional profile of larvae from experiment I, in order to test the potential of alternative protein sources and added supplements in juvenile on growth diets.

Results and discussion

Experiment 1 underlines the impact of cannibalism on survival and nutrition in H. gammarus larviculture.Analysed H. gammarus zoea 1 composition, identified deficiencies in ash and carbohydrate in lobster feeds. This suggests a need for a species-specific, formulated dry feed for H. gammarus larviculture. This experiment represents the first investigation of H. gammarus larval composition and dietary requirements and highlights decreased growth potential associated with providing nutrition solely from generic commercial feed. In experiment II, the high survival and growth, low incidence of moulting problems and high availability of waste shrimp material, suggest that non-heat-treated shrimp products are a promising feed ingredient for post-larval European lobsters.

In conclusion, this presentation has highlighted the technical, nutritional and practical innovations that have taken place over the last two decades that have allowed European lobster hatcheries to proliferate throughout Europe. However to reach a sustainably produced plate sized product that can contribute towards a local nutritious food production systems, further innovations revolving around improved feeds for all life stages, technical and ecological scale ups, systemic support and regulations and improving welfare practices are needed.

CUMULATIVE IMPACTS OF CLIMATE CHANGE AND INCREASED AQUACULTURE PRODUCTION IN A NORWEGIAN FJORD

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Introduction

The Norwegian coastal marine ecosystem is highly productive due to nutrient release from land, high incoming solar radiation (in summer) and a blend of mixing and stratifying processes in the water column. Nitrogen (N) and especially phosphorus (P) are essential elements and often the limiting factors for phytoplankton growth. However, eutrophication through N and P enrichment from human activities can stimulate the growth of algae and change the structure and function of the phytoplankton community. A planned 5-fold increase in aquaculture production will further add nutrients to the coastal systems. In addition, global climate change will impact coastal systems in a number of ways, and cumulative impacts from climate change and human activities are to be expected. Several climate pressures to the Norwegian coastal ecosystem are already in play: surface temperatures have increased by 0.7-1.3°C and increased runoff from land has contributed to increased light attenuation and "darkening" of the Norwegian coastal watermasses. Thus, the main objective of the study is to investigate the cumulative impacts of climate change, coastal water darkening and eutrophication from fish farming on lower trophic level production in a coastal ecosystem, exemplified by the Hardangerfjord at the west coast of Norway.

Methods

A coupled 3D physical, chemical and biological high resolution ocean model for plankton and nutrient cycling has been applied (Aksnes et al. 1995; Skogen et al. 1995, Albretsen et al. 2011). Five different experiments have been performed to project both the isolated and combined impacts of fish farming (nutrient release), darker water (increased light attenuation) and climate change (temperature increase) on the timing (onset of spring bloom) and amount of phytoplankton production for the example years 2018-2020.

Results and Discussion

Depth integrated modelled net annual primary production for the reference simulation without any stressors is 117 $gC/m^2/year$, with spring bloom starting at March 13. Adding estimated present day nutrient release from fish farming does not change the timing but increases the production slightly (5%), while increased temperature accelerates the spring bloom by 3 days and reduces the productivity by only 1%. The combined effect of present-day fish farming and temperature increase is 4% increase in production and 3 days earlier spring bloom, while the largest change in production is seen from the combined effects of a 5-fold increased fish farming together with temperature increase. This causes a major increase in production (20%) and 3 days earlier spring bloom. Reduced light levels, however, have an opposite effect on timing and delays the spring bloom by 4 days, and reduces the production by 6%. The cumulative net effects of all three pressures (increased fish farming and temperature, reduced light in the water column) are no change in spring bloom start, though the primary production in the area still increases by 9%

Our results suggest that eutrophication from increased fish farming in the Hardangerfjord will increase the primary production levels in the fjord substantially, though the total effect might be dampened by coastal water darkening. The ecological consequences for a future fjord are, nevertheless, largely uncertain. One potential effect is that increased amounts of phytoplankton may stimulate zooplankton growth and thus provide an extra food resource for fish. However, both temperature increase and reduced light levels are expected to shift the phytoplankton community towards smaller sizes, which may alter the dynamics and energy transfer between phytoplankton and zooplankton. Furthermore, other studies have shown how the combined effect of eutrophication and reduced water clarity can lead to ecological regime shifts, going from fish- to jellyfish-dominated ecosystems. These potential consequences should be addressed by future studies to enhance our understanding of how coastal ecosystems might be affected by a changing climate and growth in the coastal aquaculture sector.

Conclusion

In the Hardangerfjord, increased nutrients from a 5-fold increase in farming activities in a warming climate can induce an as high as 20% increase in primary production. The accumulated effect of higher temperature, darker water and increased nutrient loads will altogether result in a 10% increase in primary production. The ecological effects of the changes remain to be studied.

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DICLOFENAC, IBUPROFEN AND MIXTURE THEREOF CAUSED OXIDATIVE STRESS AND INCREASE OF HSP70 IN RAINBOW TROUT

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequent and most common pharmaceuticals used in medicine due to their analgesic, antipyretic, and anti-inflammatory effects. Their use on daily basis resulted in NSAIDs have a leading position among the pharmaceutical contaminants present in the aquatic environment. Even so, their complex mechanism of toxicity for fish is not fully understood. We selected rainbow trout (*Oncorhynchus mykiss*) as an ideal model to examine the impact of two NSAIDs – diclofenac (DCF) and ibuprofen (IBP). The aim of our study was to test toxicity via dietary exposure of environmentally relevant concentrations (2 μg kg^{-1}) of these drugs together with exposure doses of 100× higher (200 μg kg^{-1}), including their mixture (combination of 2 μg kg^{-1} DCF and 2 μg kg^{-1} IBP). Here, we present the results of oxidative stress markers and gene expression.

Materials and Methods

We performed a test in accordance with OECD 215 methodology (Fish Juvenile Growth Test) in which DCF, IBP and their mixture were incorporated into commercial feed pellets. A total of 144 individuals were divided into control and five experimental groups fed by DCF in c = 2 μg kg^{-1} (DCF2) and 200 μg kg^{-1} (DCF200); IBP in c = 2 μg kg^{-1} (IBP2) and 200 μg kg^{-1} (IBP200), including their combination, i.e. DCF in c = 2 μg kg^{-1} mixed with IBP in c = 2 μg kg^{-1} (DCF2+IBP2). The experiment lasted six weeks and ended by taking the biometric data, blood and samples of tissues for subsequent laboratory analyses. Hodkovicova et al. (2022) refer in detail all of the methodological and statistical procedures of sample and data processing.

Results and discussion

The kidneys, liver and gills were discovered to be the most severely affected tissues in our experiment and in all of them, the outbreak of reactive oxygen species (ROS) was observed. In posterior kidney, the environmentally relevant DCF concentration caused an increase in the catalase (CAT) activity – an enzyme which major function is to eliminate hydrogen peroxide as one of the ROS representatives. In liver, marker of lipoperoxidation (TBARS), increased in DCF2 and IBP200, as well as in DCF2+IBP2 combination. Regards gills, we confirmed the decreased glutathione peroxidase (IBP2, IBP200 and DCF2+IBP2) and CAT activity (DCF200). In general, ROS can oxidise cellular macromolecules, i.e. lipids, proteins and DNA, and provoke cell death.

The heat shock protein 70 (*hsp70*) is a commonly used marker for assessing the impact of environmental pollutants and its mRNA levels are known to increase in response to various types of stress. *Hsp70* is also an important factor in protein function, maintaining cell homeostasis, and affecting cytokine production. Regards *hsp70* expression, the environmental concentration of IBP caused an increase in the anterior kidney and liver. In gills, the *hsp70* expression increased in both tested concentration of IBP.

In conclusion, due to changes in activity of antioxidant enzymes, we assume that the toxicity of NSAIDs in rainbow trout manifests itself mainly through their effect on the function of mitochondria. Damage of mitochondria can lead to increased ROS and cell death; consequently, the ROS increase can provoke a proteotoxicity that reflected by *hsp70* increase in kidney, liver and gills. We kindly invite you to take a look at our poster and our study results (Hodkovicova et al., 2022), where the detected changes are presented in a comprehensive overview.

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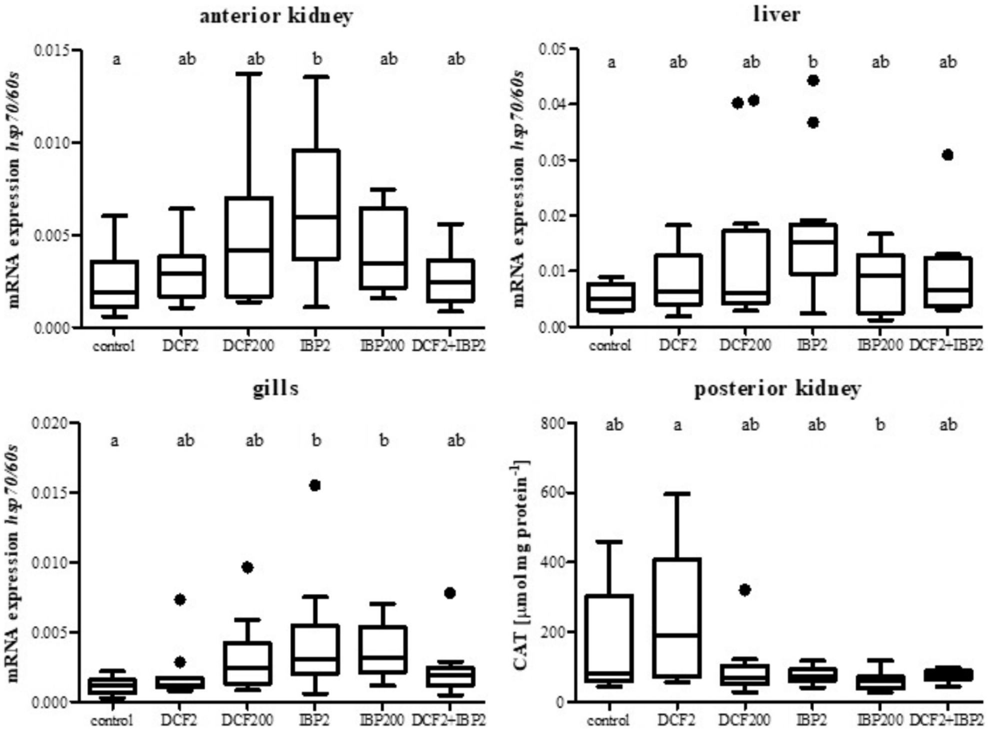
Figure 1. The *hsp70* mRNA expression and the catalase (CAT) activity in tissues of *O. mykiss* after six weeks of oral exposure to diclofenac and ibuprofen in concentrations 2 and 200 μg kg^{-1} (DCF2, DCF200; respectively IBP2, IBP200); including their mixture (DCF2+IBP2). Significance was stated with different letters of superscript (n= 8).



Acknowledgments

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NMR-BASED METABOLIC PROFILE IN MUSCLE OF JUVENILE TURBOT (*Scophthalmus maximus*) FED WITH PLANT AND ANIMAL PROTEIN SOURCES

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Introduction

The reduction of the environmental footprint of diets for farmed fish that are nutritious but at the same time economically and environmentally sustainable has become a high priority (Naylor et al. 2021, Glencross et al. 2020). Turbot (*Scophthalmus maximus*), a high-value species in Europe, has a high potential for sustainable production and is suitable for diets with novel ingredients. Although alternative feed ingredients such as insect meal or processed animal protein have no or little effect on growth and feed performance, nutritional status indicators were negatively affected at low inclusion levels (Hoerterer et al. 2022). Using ^{1}H-nuclear magnetic resonance (NMR) spectroscopy, this study examined the diet dependent effects on the metabolic response in muscle tissue of juvenile turbot after the fish were fed with different diets formulations including plant and animal protein sources.

Material and Methods

In a 16-week feeding trials with 750 juvenile turbot (20 ± 0 g), two formulation concepts were tested against a commercial formulation (CTRL). The alternative formulation concepts were based on replacing 20 % of fishmeal with insect meal and fermentation biomass. Furthermore, sustainable terrestrial plant proteins (NoPAP) or processed animal proteins (PAP) replaced the soybean content in the CTRL (see figure 1). The metabolic profile of a perchloric acid extract of the muscle and liver tissue of N = 45 juvenile (n = 15 per diet) was generated using ^{1}H NMR spectroscopy (Rebelein et al., 2018). A Partial Least Squares - Discriminant Analysis (PLS-DA) of the metabolic profiles was performed to identify differences between the formulation concepts using MetaboAnalyst 5.0

Results and Discussion

The comparative approach revealed that metabolites associated with growth and energy allocation showed different levels in the muscle of fish fed with the alternative formulation concepts (NoPAP and PAP) and the commercial formulation (CTRL). Creatine levels were highest in fish from the CTRL group indicating fast energy provision in the body and good muscle growth. Furthermore, taurine levels were highest in muscle tissue of fish from the PAP group indicating that the dietary taurine (supplemented with 0.5 % in NoPAP and PAP) was metabolised better than in fish from the NoPAP group (see figure 2).

In general, the results from the ^{1}H NMR generated metabolic profiles and following PLS-DA indicated that metabolites such as taurine, creatine, acetate and glutamine are diet-dependent in muscle tissue of turbot and that the NoPAP and PAP formulation concepts might affect the taste and consistence of muscle tissue of the final consumable product. More insight on the diet dependent effects on the energy allocation in turbot will bring the analysis of the liver of the juvenile turbots. Sensory analysis of the filets might confirm the NMR-based metabolite findings in the muscle.

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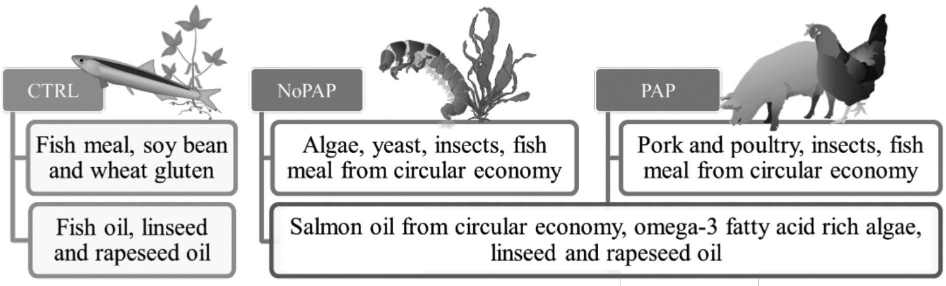

*Figure 1. Formulation concepts of the experimental diets: CTRL: commercial formulation, NoPAP: no processed animal protein included, PAP: with processed animal protein (*pictures: ian.umces.edu/symbols/)

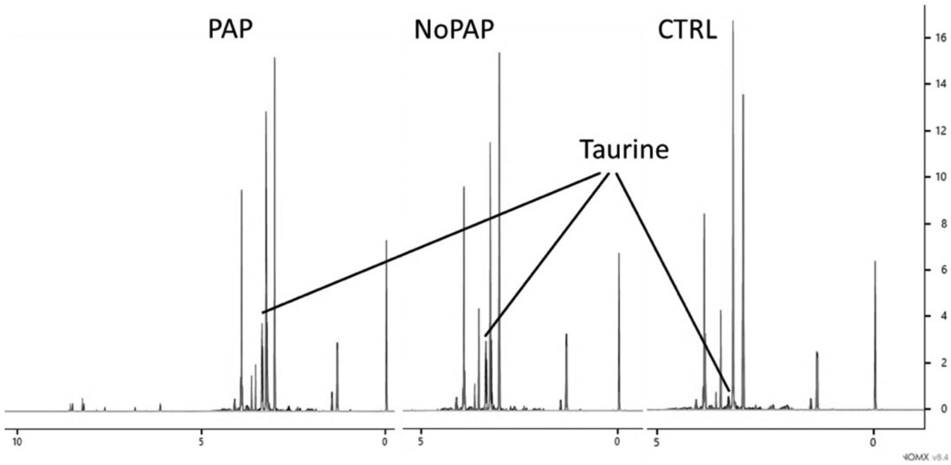

Figure 2. Exemplary ^{1}H NMR spectroscopy spectra of perchloric acid extracts *of muscle tissue from juvenile turbot (n=15 per diet) fed with commercial formulation (CTRL) and the two experimental formulation concepts (NoPAP and PAP). (spectra generated with CHENOMIX v8.4).*

APETITE REGULATED FEEDING BY AUTOMATED FEED WASTE MONITORING IN CLOSED AQUACULTURE SYSTEMS (CAS)

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Introduction

In precision feeding (PF) the feeding is automatically regulated by the appetite of the fish. In closed aquaculture systems (CAS), landbased and sea based, there is a physical barrier between the fish and the surrounding environment, which enables development of technologies for appetite regulated feeding. By monitoring uneaten feed in the waste drain, information about the appetite is obtained. This information can then be used to regulate the amount of feed that should be added to the tank at any time.

Automated feeding is an important step towards sustainable aquaculture as it has a great impact on many different aspects of the industry.

In CAS almost all suspended solids originate from added feed, either directly as uneaten feed or indirectly as feces. A smaller portion by mass comes from biofilm shedding and biofloc (dead and living bacteria) (Timmons et al., 2018). In terms of water treatment efficiency and water quality stability, controlling the solids tightly through PF is very important. High levels of suspended solids increase heterotrophic bacterial growth, which in turn can reduce TAN removal efficiency in RAS and deteriorate water quality (Chen et al., 2006). It has been stated that suspended solids in RAS should be kept below 15 mg/l (Thorarensen and Farrell, 2011).

In terms of growth, feeding according to appetite can improve the feed conversion ratio (FCR) because of the reduced underfeeding. This directly impacts the production capacity as well as the predictability for the farmer. Feeding according to appetite also improves the fish welfare as feed will be offered whenever the fish is hungry.

Furthermore, PF is an important step by reducing wasted feed in the industry and reducing the cost for the farmer as less feed is lost. Feed is also the main contributor to CO_2 emission in aquaculture. Controlling the feed will therefore also reduce the CO_2 footprint.

The results from PF will be significant as the production will be more predictable and less risky, and potentially enable industry growth in CAS.

Method

A system for Precision Feeding (PF) and feeding according to appetite is under development. The technology is based on a patented camera-based sensor system mounted in the waste drain. The camera monitors feed waste, and the observations are interpreted to build predictive models and system simulations that can be used for regulation of feeding, either as decision support or as fully automated actuation.

Monitoring wasted feed in CAS can be a challenge as the color of the water can be dark and the number of particles and level of turbidity high. The camera-based sensor system for monitoring feed waste needs to operate under these challenging water quality environments. Under these conditions the camera needs to see the difference between feed and faeces. For that the camera needs to be highly light sensitive and the external light needs to have the correct wavelength and positioning (angle).

Several tests have been conducted in different experimental conditions, both in research recirculating aquaculture systems (RAS) with fish and in test labs without fish.

Results

The experimental systems were successfully achieved. The results are under investigation and will be presented at the conference.

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THE EFFECT OF ORAL MICROPLASTICS AND FLAME RETARDANTS APPLICATION ON THE SELECTED GENES EXPRESSION OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Plastic pollution is a global problem caused by an excessive use of this material, high resistance in environment and poor waste management. One of the most common type of plastic polluting the environment is polystyrene (PS), which commonly contains flame-retardant, hexabromocyclododecane (HBCD), in high percentage. In general, oral ingestion of MPs can cause mechanical damage and obstruction of digestive tract and particles < 600 µm are able to cross intestinal barriers, enter the blood stream and can be transported to tissues. While in vertebrates, the toxicity of HBCD has been extensively studied with discovered reproductive and developmental toxicity, neurotoxicity, mutagenicity, as well as endocrine disrupting effects, the mechanism of toxicity is not fully clarified in fish to date. This study was focused on the analysis of genes via gene expression, which will be the basis for further analyses.

Materials and methods

The experimental part of the study was carried out in accordance with Fish Juvenile Growth Test (OECD 215) in duplicate. A total of 144 *O. mykiss* juveniles were divided to control and groups exposed to 2%PS (in size 200-300 µm), 1%HBCD and mixtures of 2%PS+ 0.1%HBCD and 2%PS + 1%HBCD of daily feed intake. The experiment was terminated after six weeks. For gene expression analysis made by quantitative real-time polymerase chain reaction method, the samples of liver, skin and cranial intestine (n=10 per group) were processed according to Hodkovicova et al. (2021). Selected genes were interleukin 8 *(il8)*; catalase *(cat)* and cytochrome P450 1a3 *(cyp1a3)*.

Results and discussion

During the experiment lasting, the expression of *il8* decreased in the group exposed to 2%PS-MPs in the liver and cranial intestine (Figure 1).

Cytokine *il8* is produced mainly by monocytes to recruit neutrophils as well as trigger cytotoxic effect in site of infection. Hodkovicova et al. (2021) observed *il8* up-regulation in the liver and cranial kidney of rainbow trout exposed to polyethylene MPs. In this case, uncontrolled proliferation with potential to participate in chronic diseases can occur. Our study showed the opposite results, i.e. - *il8* down-regulation by 2%PS, which can affect the immune system by limitation of neutrophil function (Wang et al., 2017). This demonstrate the various effect of different types of MPs and need for further investigations. In addition, in liver tissue the expression of *cat* increased at 2%PS. In the skin, the expression of *casp3* increased in 2%PS + 0.1%HBCD, *cyp1a3* increased in 2%PS + 0.1%HB CD, also in 2%PS + 1%HBCD in compared to control. The results are expressed in figure 2.

Catalase is antioxidant enzyme and marker of oxidative stress that breaks down hydrogen peroxide into oxygen and water. Increased *cat* expression is likely a response to toxic stress triggered by PS-MPs and serve to neutralize the increased reactive oxygen species production (Kumari et al., 2014). Cytochrome 1a3 belongs to P450 superfamily of enzymes which catalyse oxidative, peroxidative, and reductive xenobiotic metabolism. In addition, combination of PS and HBCD caused up-regulation of *cyp1a3* in skin by which we hypothesize that molecular detoxifying mechanisms was activated (Bertucci et al., 2020). In summary, prolonged weakening of liver, intestine and skin can cause severe health disruption and increase fish mortality. Histopathological examination and analysis of oxidative stress will be made to verify our hypothesis for mechanism of toxicity provoked by PS and its common additive, HBCD.

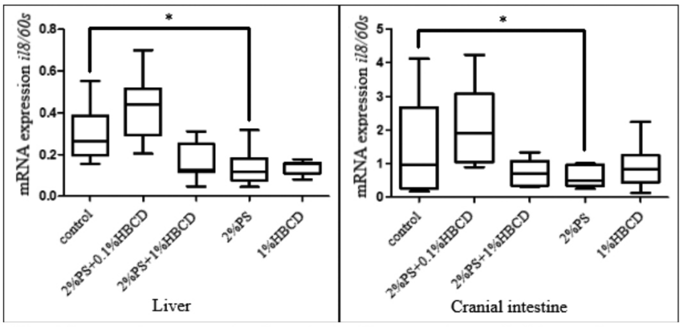

Figure 1. Significant decrease in the *il8* in liver and cranial intestine. Results with significance are marked with * ($p<0.05$).

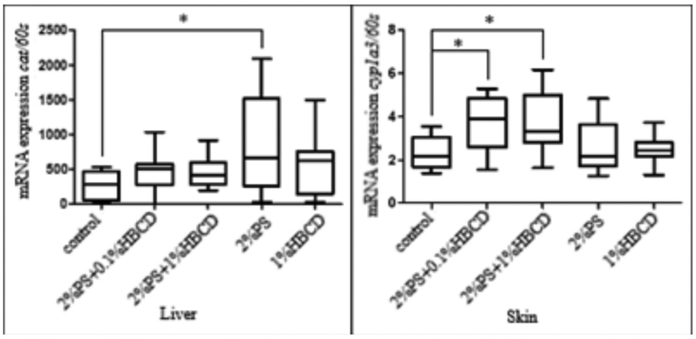

Figure 2. Significant differences in the expression of *cat* in liver *cyp1a3* in skin. Results with significance are marked with * ($p<0.05$).

Acknowledgments

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MOLECULAR CHARACTERIZATION OF DIFFERENT REPRODUCTIVE GENES OF THE HYPOTHALAMIC-PITUITARY-GONADS AXIS OF THE DUSKY GROUPER *Epinephelus Marginatus* (PERCIFORMES: SERRANIDAE)

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Introduction

Epinephelus marginatus (Dusky grouper) is an endangered species with great potential for aquaculture in Brazil (Rodrigues-Filho et al., 2020). On the other hand, when kept in captivity, this hermaphrodite protogynous species presents reproductive dysfunctions, reducing the success of its large-scale rearing (Moreira et al., 2019). Nowadays, we are designing specific *primers* for this species to perform rtPCR and analyze the gene expression of the main components of the Hypothalamus-Pituitary-Gonads (H-P-G) axis to better understand the origin of these reproductive dysfunctions in captivity animals.

Material and Methods

In the present study, two groups were considered during the reproductive period and outside of the reproductive period: animals raised in captivity (G1 – as experimental group) and animals sampled from natural environment (G2 – as control group). Brain, pituitary, and gonads of fish from both groups at different moment were sampled for gene expression study. We analyzed the gene expression of brain aromatase (*cyp19a1b*), and the *gnrh* (three distinct forms), both in the brain; gonadotropins (*fshb* and *lhb*) were analyzed in the pituitary; and several genes involved in the sexual differentiation and steroidogenic pathway (such as, *sox9, dmrt1, amh, foxl2, 20β-hsd,* and *cyp19a1a*) were also analyzed in the gonads. Additionally, the genes *ef1α* and *cog5* were used as endogenous references for the analysis of the relative expression of target genes. The samples were processed to conventional PCR for DNA amplification of their respective target genes. Then, using primers developed and patterned for other species of *Epinephelus* genus, we sequence the DNA from PCR products by DNA Sequencing – SANGER. For brain (*gnrh* 1, 2, and 3 and *cyp19a1b*) and gonads (*sox9, dmrt1, amh, foxl2, 20b-hsd,* and *cyp19a1a*), we are designing by sequencing the DNA fragment that bounds to the primers and testing new specific primers for *E. marginatus*. Moreover, gonadotropins, *fsh* and *lh*, and *ef1a* have been already performed and apparently were specific for *E. marginatus* by our research group.

Results

It is expected that the results of this study will show whether the endocrine disruption observed in animals kept in captivity is due to changes in the gene expression of neurohormones, neurotransmitters, gonadotropins, and gametogenesis regulators, which coordinate the H-P-G axis. Additionally, these primers will be specific for this species, and it is the first time that they will be described for *E. marginatus*. In Figure 1 we presemt the first results of conventional PCR for these H-P-G gene target of *E. marginatus*.

Final considerations

Previous studies conducted by our research group suggest that *E. marginatus* presents reproductive dysfunction when kept in captivity (Rodrigues-Filho et al., 2020), mainly in the gonadal maturation of females. In these previous studies, it seems that dysfunctions in the gene expression of different targets of the H-P-G axis must occur, primarily in gonadotropins expression (Garcia et al., 2013). All this information is important to improve the artificial reproduction of *E. marginatus* through controlled breeding programmers, considering the threatened situation of this species, that requires immediate recommendations to be taken, to avoid the extinction of this important species. Additionally, these results may support future studies on Brazilian aquaculture of marine teleost species.

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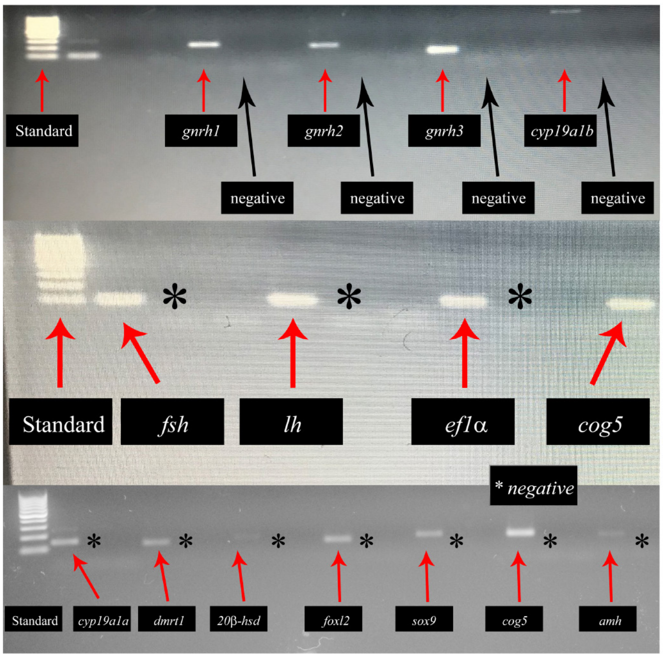

Fig1. Results of conventional PCR of the main components of the Hypothalamus-Pituitary-Gonads axis in *Epinephelus marginatus*.

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Acknowledgments

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STARCH – AN ANTINUTRITIONAL FACTOR FOR YELLOWTAIL KINGFISH *Seriola lalandi*?

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Introduction

Yellowtail kingfish (*Seriola lalandi)* is a fast growing fish species. One of the challenges of farming yellowtail kingfish in recirculating aquaculture systems (RAS) is their poor faecal quality, also referred to as 'diarrhoea-like' faeces. Poor faecal quality hampers the faecal solid removal, causing high concentrations of total suspended solids (TSS) in the water. This affects the animal health, system performance and environmental eutrophication negatively (Reid et al., 2009). A study with juvenile white sturgeon (*Acipenser transmotanus*) by Hung et al. (1990) implies that starch level can negatively affect osmolality, water reabsorption and thus potentially faecal characteristics. Therefore, the current study investigated whether the inclusion of starch induces a poor faecal quality in yellowtail kingfish. This was done by comparing faecal quality and production of yellowtail kingfish fed diets varying in starch inclusion and diet type.

Materials and Methods

The effect of starch inclusion (0% // 20% gelatinized wheat flour) on faecal waste production and quality of yellowtail kingfish was investigated for two diet types (marine // plant-based) over a 36-day experimental period. Diets were formulated according to a 2 × 2 factorial design. Each dietary treatment was tested in three replicate tanks, which were stocked with 21 yellowtail kingfish (mean initial weight 53 g). Fish were fed restrictively ($23.75\ g/kg^{0.8}$, expected FCR 0.9) twice a day. For each tank, nutrient digestibility and faecal recovery by settling was measured.

Results

Both the inclusion of starch and plant-ingredients negatively affected the nutrient digestibility, thus resulting in an increased faecal waste production (g organic matter/kg feed intake) ($p < 0.001$; Figure 1a). The faecal quality of yellowtail kingfish fed 0% starch diets was not poor (not-diarrhoea-like; Image 1) as fish excreted faecal pellets and short strings. However, faecal waste collected from fish fed 20% starch diets was classified as diarrhoea-like. Fish fed the 0% starch diets had the highest faeces recovery (%) by settling compared to the diets without starch inclusion ($p < 0.001$; Figure 1b). Consequently, the non-recovered amount of faeces (g organic matter/kg feed intake) at the 0% starch diets was on average 65.5% lower compared to the 20% starch diets ($p < 0.001$, Figure 1c).

Conclusion

Excluding starch from the diet of yellowtail kingfish reduced the faecal waste production and improved quality (not diarrhoea-like), ultimately resulting in a lower amount of non-recovered faeces compared to 20% starch diets. Thus, excluding starch from yellowtail kingfish diets will result in a reduced TSS load in RAS, ultimately improving animal welfare, system performance and reducing the environmental eutrophication. Moreover, this findings offer new possibilities for other finfish species with a poor faeces quality such as Atlantic salmon (*Salmon salar*).

Results

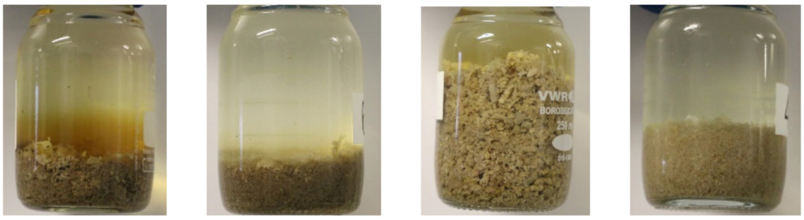

Image 1. *Collected faeces of yellowtail kingfish fed the marine 0% starch, marine 20% starch, plant 0% starch and plant 20% starch diets (left to right) restrictively for 36 days.*

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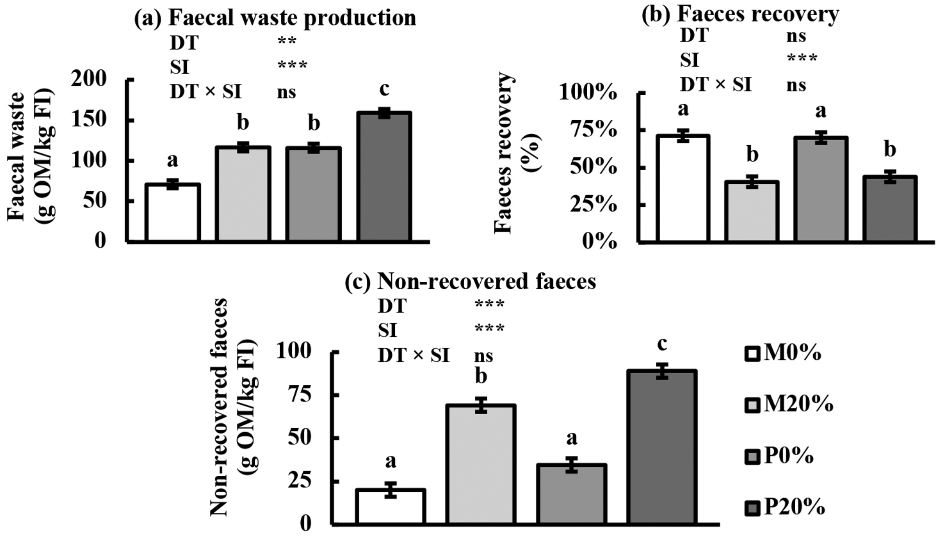

Figure 1. *(a) Faecal waste per feed intake (g OM/kg FI), (b) Faeces recovery by settling (%) and (c) Non-recovered faeces per feed intake (g OM/kg FI) of yellowtail kingfish fed the experimental diets restrictively (3 replicates) for 36 days; OM – organic matter; FI – feed intake; M0% - marine 0% starch; M20% - marine 20% starch; P0% plant 0% starch; P20% - plant 20% starch; DT – diet type; SI – starch inclusion; DT × SI – diet type × starch inclusion; bars are means; error bars indicate standard error of means; bars not sharing a common letter are significantly different ($p < 0.05$); ns – not significant; ** - $p < 0.01$; *** - $p < 0.001$.*

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CRYOPRESERVATION AND TRANSPLANTATION OF GERMLINE STEM CELLS IN SALMONIDS: WHAT HAVE WE LEARNT?

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Introduction

The need for conservation of genetic resources of fish leads to a continuous development of methods in reproduction. Cryopreservation of gametes offers a solution for the long-term preservation of these resources. In fish, however, only sperm can be cryopreserved which puts the application of these methods in disadvantage. To overcome this problem, a set of methodologies have been developed for the cryopreservation and transplantation of germline stem cells (GSCs) such as spermatogonia and oogonia for the surrogate production of gametes (Yoshizaki et al., 2011). In the present work, we describe the results of 7 years of experiments on the cryopreservation and interspecific transplantation of salmonid GSCs.

Materials and Methods

The studies have been carried out in Slovenia and Hungary. Brown trout (*Salmo trutta m. fario*), grayling (*Thymallus thymallus*) and rainbow trout (*Oncorhynchus mykiss*) were used as donors of GSCs whereas the hybrid tiger trout (*S. trutta m. fario* × *Salvelinus fontinalis*) as well as diploid and triploid rainbow trout were used as recipients.

Brown trout ovarian tissue was vitrified in varying concentrations of methanol, dimethyl-sulfoxide (DMSO) and propylene glycol (PG). Isolated GSCs of brown trout and grayling were transplanted into diploid and triploid rainbow trout larvae whereas those of the rainbow trout were transplanted into larvae of the hybrid tiger trout. Colonization of the recipient gonads as well as incorporation of donor-derived GSC-s was verified using fluorescent microscopy, molecular biology and histology.

Results

Vitrification of salmonid ovarian tissue using needle-immersed vitrification (NIV) on acupuncture needles using 3 M DMSO and 3 M PG as cryoprotectants resulted in up to 40% survival of cells and qualitatively and quantitatively unaltered follicles (Lujić et al. 2017). Transplantation of brown trout and grayling GSCs into diploid rainbow trout recipients resulted in successful colonization of 26-28% of the recipient gonads. This was verified by fluorescent microscopy and the presence of donor-specific molecular markers 2 months post-transplantation (Lujić et al. 2018). In the trials using the hybrid tiger trout as a recipient, one male individual has reached sexual maturity 22 months post-transplantation and produced sperm that was used to fertilize rainbow trout eggs. The resulting progeny was verified to be 100% rainbow trout using sequencing of species-specific DNA fragments. On the other hand, hybridization resulted in very high mortalities (up to 95%).

Transplantation of brown trout and grayling spermatogonia into triploid rainbow trout recipients was conducted in the form of 3 independent trials in Slovenia and Hungary. The recipients failed to reach sexual maturity and were sacrificed 3 years post-transplantation. Their gonads were underdeveloped and typical of triploid fish. Brown trout- and grayling-specific fragments of the mitochondrial DNA control region, however, were amplified in all three trials in the gonads of several recipients.

Discussion

The results of our studies have shown the importance of finding the appropriate combination of donor and recipient in salmonids. It is clear that brown trout and grayling GSCs were capable of colonizing the gonads of rainbow trout recipients, however, in triploid individuals they remained in a dormant or semi-dormant state and did not undergo gametogenesis. Our findings are in contrast with those of Hattori et al. (2019) who were able to obtain donor-derived sperm and eggs from triploid rainbow trout recipients following transplantation of Atlantic salmon (*Salmo salar*) spermatogonia. The hybrid tiger trout was able to produce donor-derived sperm, however, its use as a recipient was compromised by the high mortality of the hybrids. In addition, some tiger trout males are known to produce sperm which further complicates their utility as recipients.

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Acknowledgements

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IMPACTS OF CLIMATE CAUSED SALINITY INSTRUSION ON EARLY DEVELOPMENT AND GROWTH PERFORMANCE OF STRIPED CATFISH

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Introduction

Climate changes induced salinity intrusions in the coastal areas are cause for serious concern in tropical and subtropical countries (Rabbani et al., 2013). Efficient management of brackish water environments is needed to overcome the climate-caused obstacle. Striped catfish (*Pangasianodon hypophthalmus*) is a popular freshwater aquaculture species. Its growth rate is high and grows well in higher density and this fish can survive in a wide range of environmental conditions. Embryos and larvae are very sensitive to any changes during all stages of fry production (Akitas et al., 2004; Shahjahan et al., 2017; Rahman et al., 2020; Ashaf-Ud-Doulah et al., 2021). So it is important to examine the influence of salinity on embryonic and larval specimens to understand the likelihood of survival, growth, and physiological stability of this species throughout their life cycle in coastal aquaculture systems. The present investigations may provide insights into the optimum salinity level for successful egg incubation and early development, which might be helpful to produce fingerlings in brackish water for the sustainable coastal aquaculture of this species and can potentially be used as a candidate species for aquaculture in slight brackish water areas of Bangladesh as it can tolerate definite range of salinity.

Materials and methods

Striped catfish embryos and larvae were exposed to seven salinities (0, 2, 4, 6, 8, 10 and 12 ppt) in triplicate. Fertilized eggs (n= 300) and larvae (n=300) were exposed in different salinities. The number of dead embryos and larvae were counted at 24 hour and 48 hour of salinity exposure respectively. Hatching rate and larval mortality were recorded. Malformations of embryo and larvae were observed under digital microscope and images were snapped. To know the salinity effects on growth performance of striped catfish (*Pangasianodon hypophthalmus*) firstly, median lethal concentration (LC_{50}) of salinity for striped catfish was determined and then the fish were exposed to three salinity conditions (4, 8 and 12‰) and a control (0‰). Fish were counted and weighed at 7, 14, 28, and 56 days after start of salinity exposure. Growth performance and feed efficiency were determined by evaluating a number of growths and nutrient utilization indices, such as, weight gain, percent weight gain, specific growth rate (SGR) and feed conversion ratio (FCR).

Results

A considerable survivability of embryos was recorded up to 6 ppt. Mortality of embryos significantly increased at 8 and 10 ppt and 100% mortality was displayed within 12 h of exposure at 12 ppt. The rate of hatching was significantly reduced at 8 and 10 ppt. Different types of deformities were highest at 10 ppt. Similar to embryo, a considerable survivability of larvae was recorded up to 6 ppt and 100% mortalities were found within 24 h of exposure at 12 ppt and deformities were highest at 10 ppt after 48 h of exposure (Figure 1). Within 24 h of exposure about 80% yolk-sac of the larvae was absorbed at 8 and 10 ppt while 30-50% yolk-sac was absorbed at 0 to 6 ppt. Growth rates in terms of length and weight were higher at 0, 2 and 4 ppt, and moderate at 6 and 8 ppt. Salinity levels from freshwater to 8‰ showed optimal conditions with high survival rate and good growth performances of fish in terms of weight gain and specific growth rate (SGR). Interestingly, the lowest food conversion ratio (FCR) was found in 4‰ group.

Conclusion

Salinity is among the most important environmental factors that influence the physiological processes of aquatic organisms. Overall, the current findings define the limits in order to optimize hatchery procedures for culture of this species in low saline brackish water and revealed that striped catfish might be suitable fish species for culture in the brackish water containing salinity up to 10‰.

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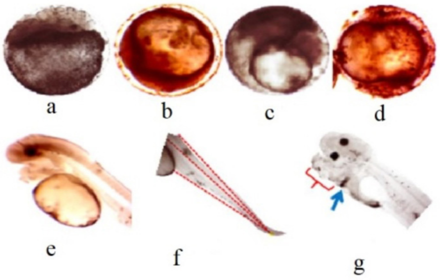

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EFFECTS OF MULTI-SPECIES PROBIOTICS ON GROWTH AND GUT HEALTH OF NILE TILAPIA

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Introduction

Aquaculture is considered as one of the most competitive and promising sectors for providing income and nutritional security to human, as demand for animal protein from fish is growing globally (Mohapatra et al., 2012)T 2 (BF+Bacillus subtilis and Lactococcus lactis. Although the sector has achieved remarkable improvement, in recent time the income of this field is declining because of huge feed costs that account for over 70% of total production expenses (Hossain et al., 2020, Nguyen et al., 2020). Use of probiotics may be an ideal approach for better feed utilization and growth enhancement in fish reducing the feed cost as well as substituting use of antibiotics (Allameh et al., 2017). Thus, the goal of this study was designed to see the effects of multi-species probiotics in water on the growth, and intestine, liver and muscle morphology in the Nile tilapia.

Materials and Methods

Tilapia fingerlings (5.95 ± 0.07 g) were stocked and evenly distributed into three replicates separated into three treatment groups, each providing probiotics at concentrations of 0, 0.5 and 1.0 mL/L in water for 8 weeks. After completion of the trial, growth factors (weight gain, % weight gain, specific growth rate, hepato-somatic index, and viscera somatic index), feed utilization parameter (feed conversion ratio), gut microbiota, and intestinal, liver and muscle morphology were examined.

Results

The experiment revealed dramatic growth and feed utilization in the fish reared with multi-species probiotics compared to control group. Total viable count (TVC) and total lactic acid bacteria (LAB) count increased substantially in the fish gut provided with multi-species probiotics. The morphological improvement of intestine includes increased intestinal length, width, and area of the villi, lamina propria and abundance of goblet cells were observed in the probiotics treated fish (Figure 1). The livers of treated fish showed irregular shaped nucleuses turned into regular shaped and reduced spaces between liver tissues. Increased number of nucleus, diameter of muscle fiber, hyperplastic muscle fiber was also found from morphological observation from the multi-species probiotics applied fish compared to control group. The study indicates administration of multi-species probiotics can be an important growth promoter for Nile tilapia.

Conclusion

Multi-species probiotics administration in water demonstrated supreme growth and feed utilization of Nile tilapia through enhancing gut microbial content, and gut, liver and muscle morphology. Further investigation on probiotic is required to know the relative expression level of muscle developmental related genes, different enzymatic activity, disease resistance and which types of response is occurred due to probiotic exposure on host species. Furthermore, which types of bacterial strain are responsible for specific improvement for specific organ is needed for multi-species probiotic.

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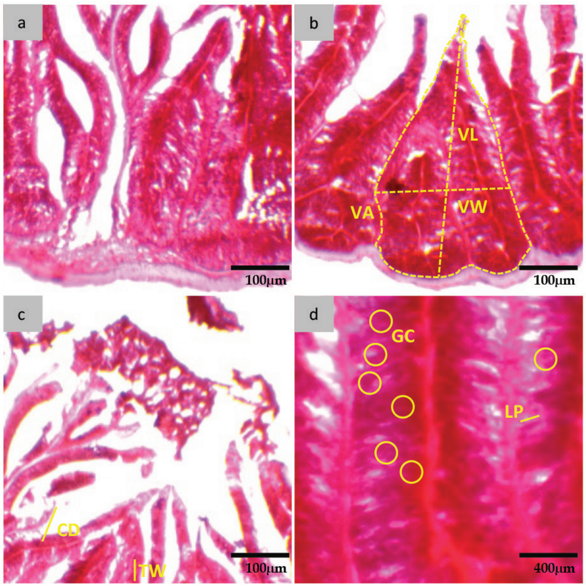

Figure 1: Histological observation of intestine of Nile tilapia given with probiotic supplemented with water (a) 0 mL/L; control and (b-c) 0.5mL/L, & 1mL/L for 60 days: VA; Villus area, VL; Villus length, VW; Villus width, CD; Crypt depth, TW; Thickness of wall, TM; thickness of muscular, MF; Mucosal folds, LP; Lamina propria, GC; Goblet cell and EC; Enterocyte.

THE CASE FOR LAND-BASED BIVALVE FARMING

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Abstract

Current estimations suggest that over 2 billion people world-wide suffer from a micronutrient deficiency (MND), which may increase with global population size, unless current food practices change. As agricultural land is limited, and common farming practices have had detrimental consequences over the years on the environment and human health, the development of novel farming techniques has become vital. A novel onshore aquaculture approach for marine bivalve molluscs offers considerable promise for easing these global challenges. Here we explore their potential value to food security by comparing oyster, mussel, and clam nutrient profiles against top Finfish, Crustacea, and agricultural livestock nutrient profiles; reveal the gap for onshore marine bivalve farming; and set the grounds for the development of urban sourced marine bivalve aquaculture, which promises to address MNDs whilst mitigating the current human health risks associated with consumable bivalves.

Global Bivalve Production is Not Represented in Landlocked Regions

Mapping global bivalve edible weight (Figure 1) from the latest FAO fisheries and aquaculture report, 2022, reveals production of livestock is less than 1 Mt in landlocked countries. With current marine bivalve aquaculture being limited to coastlines, this confirms that there is untapped farming potential onshore within geographical locales where no coastal farming is possible. If marine bivalves are to deliver on their promise of being blue foods capable of addressing current global food security challenges and MNDs, then urban aquaculture must adapt to support the growth of marine bivalves for their ultimate consumption and possible remediation for the proportion of the population they currently do not reach. However, to allow such a system to be viable, there must also be high gross margins which are led by consumer demands. For this reason, the top produced mussel, oyster, and clam were also identified (Figure 1) to allow for an understanding of what bivalve livestock would be best to rear within an urban marine bivalve aquaculture facility.

Potential for Urban Aquaculture of Marine Bivalves

The development of a marine bivalve aquaculture system that could be implemented within the city environment, without the need for the ocean, would allow marine bivalve production to be expanded. This expansion would not only bring with it the introduction, and increased availability of a highly nutritious food source to currently excluded geographical locales (Figure 1), but such a system could also bring many benefits including MND amelioration, increased food safety, production of high value by-products, poly-culture, and the foundation to expand marine onshore aquaculture as a whole (Figure 2). However, inputs such as electricity, feed, and maintenance for an onshore facility could result in higher start-up and overall costs compared to current marine bivalve farming, which is solely ocean based (Figure 2). Though, with the development of such a system, there also comes the need for optimization to help unlock its benefits while keeping costs as low as possible for both the facility and consumer.

Conclusion & Future Directions

The present work reveals a large gap in global geographic access to fresh marine bivalves, and thus their promising role in addressing current population MNDs as well as being a highly nutritious food source. The development of a novel urban marine bivalve aquaculture system will not only set the groundwork for its translational use towards addressing global food security, but also aid in addressing fundamental questions that are still unknown in bivalve mariculture, and MND reduction within the human population.

Future work includes optimizing bivalve growth, decreasing bivalve related health hazards, optimizing sustainable feed, and expanding micronutrient fortification capabilities within an artificial setting. Elucidation of these methods will make possible the development of scalable technologies that will allow onshore urban marine bivalve aquaculture to gain consumer trust around bivalve consumption and deliver the nutritional promises marine bivalves have to offer.

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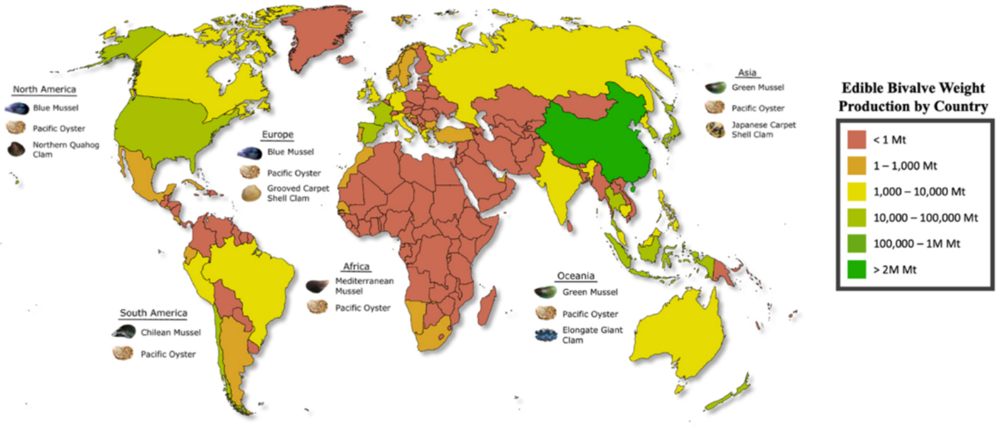

Figure 1: **Global production of edible bivalve meat and top produced oyster, mussel, and clam species.** Mapping of global edible bivalve production suggests landlocked regions and extreme temperatures lower bivalve production. Analysis of production for oysters, mussels, and clams based on continent shows ubiquitous production of the pacific oyster, with the top mussels being the green and blue mussel, and no consistent species of clam.

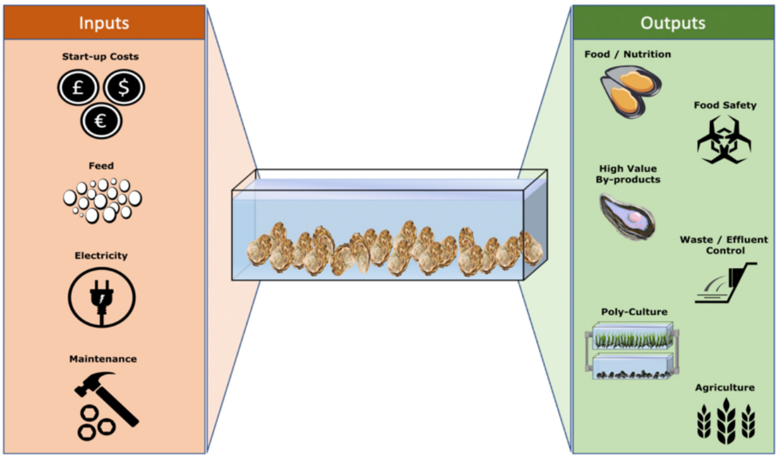

Figure 2: **Urban marine bivalve aquaculture schematic**. Development of a marine aquaculture system that can rear marine bivalves to market size requires a full understanding of the inputs required, compared to its outputs. Though there are many outputs compared to the designated inputs, the revenue produced by identified outputs must be larger than the cost of the inputs if such a system is to be successful at being a disruptive solution.

IMPACTS OF DIETARY C/N RATIOS ON WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

In recirculating aquaculture systems (RAS), good water quality needs to be maintained to ensure fish health and stable production. However, to fulfill the nutrient requirements of fish in different life stages, the proportions of carbohydrate, lipid, and protein in fish feed are continuously adjusted, which leads to different dietary C/N ratios. As fish waste excretion to some extend reflects the feed content, the amounts of C and N compounds sustained in water is expected to change as well. Indeed, dietary and water N contents are positively correlated in RAS (Godoy-Olmos et al., 2022), while the relationship between dietary and water organic matter (C) has not been examined. Undigested and non-retained dietary C and N, acting as macronutrients for microbes present in RAS, might potentially affect microbial water quality (abundance, activity, and community composition). Elevated C/N ratios in water caused compromised nitrification performance and unbalanced microbial communities in lab-scale biofilters (Michaud et al., 2014). Currently, the effects of dietary C/N ratios on water quality in RAS are still poorly understood. Such knowledge would provide foundation for cost-effective water management. The aim of this study was therefore to investigate the impacts of dietary C/N ratios on RAS water quality and microbiology at both biofilter and system levels.

Materials and Methods

COD was used as the proxy for organic carbon. By altering the proportions of protein, carbohydrate, and fat, four diets with different COD/N ratios (22.7, 24.7, 26.6, and 32.5) were formulated and termed LO (low), ML (medium-low), MH (medium-high), and HI (high) (Table 1). An 8-week trial was conducted in triplicate pilot-scale RAS situated at DTU Aqua, Hirtshals, Denmark. Each system consisted of a 500 L tank stocked with 7.31±0.04 kg of juvenile rainbow trout (*Oncorhynchus mykiss*), a moving bed biofilm reactor (MBBR) (75 L), and a pump sump (140 L). The fish in each RAS was fed 100 g/day with water renewal rate of 60 L/day (make-up water). Water samples were collected from the sump every other week, samples of biofilter elements were obtained at week 1, 4, and 8, and fecal samples were obtained by the end of the trial.

Results and Discussion

By the end of the trial (averaging weeks 6-8), the HI diet resulted in significantly lower system concentrations of nitrite (0.03±0.01 mg N/L), particulate organic matter (BOD_5, 4.89±1.16 mg O_2/L), abundance of particle-associated microbes, and microbial catalytic activity (H_2O_2 degradation k-value, 0.54±0.16/h), as well as better water clarity (9.23±2.15 NTU) than the other diets.

Overall microbial community structures in biofilter and system water did not differ between treatments, however, ammonia-oxidizing bacteria (AOB) were less abundant in biofilters with the HI diet than those with the other diets. The reduced AOB abundance was probably due to the reduced N excretion from the HI diet, leading to less substrate for nitrifying bacteria. Biofilm communities have been found to be less influenced by environmental changes such as water treatment (e.g. foam fractionation) (Aalto et al., 2022) and increase in organic carbon (Michaud et al., 2014).

Fecal COD/N ratios were subjective to dietary ratios (Table 1), which was in line with Letelier-Gordo et al. (2015). However, dietary and RAS water COD/N ratios did not correlate, mainly because water organic matter did not respond to dietary COD. Since water C/N ratios can affect nitrification performance and system microbiology, diets should be tested in RAS to examine the resulted nutrient ratios in water.

Table 1. C/N ratios of diets, water (n=6), and feces (n=3) (mean ± standard deviation): Kjeldahl N in feeds and feces, and total dissolved N in water. All units are mg O_2/mg N. Lowercase letters indicate statistical differences from the other groups.

Parameter	LO	ML	MH	HI
Dietary COD/N	22.7	24.7	26.6	32.5
Water BOD_5/N	0.11 ± 0.02^{b}	0.18 ± 0.06^{a}	0.17 ± 0.07^{ab}	0.11 ± 0.02^{b}
Water COD/N	0.72 ± 0.12^{b}	0.95 ± 0.18^{ab}	1.02 ± 0.17^{a}	0.83 ± 0.09^{ab}
Fecal COD/N	27.90 ± 1.67^{b}	35.45 ± 2.10^{a}	33.00 ± 2.09^{ab}	37.75 ± 3.29^{a}

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Conclusion

This is the first study to investigate the impacts of different dietary C/N ratios on RAS water quality. Based on the results, dietary C/N ratios could affect physicochemical water quality, microbial activity, and abundance of particle-associated microbes, while microbial community composition in system water and biofilter biofilms were not affected. Dietary and water C/N ratios did not correlate, probably because organic matter was used or incorporated in the biofilm.

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THE POTENTIAL APPLICATION OF A PARTIAL NITRIFICATION- ANAMMOX BIOFILM-BASED PROCESS FOR NITROGEN REMOVAL IN RAS

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Recirculating aquaculture systems (RAS) have lower water consumption and lower nutrient discharge to the environment than the traditional flow-through systems, making them environmentally sustainable fish-producing systems. However, waste management still need to be improved to reduce environmental impact. Partial nitrification combined with anammox (PNA), which combines the anammox with the partial nitrification, is currently applied for nitrogen removal from wastewater with very high concentrations of TAN, and is characterized by reduced requirements for aeration, no need for addition of carbon source, lower sludge production. In this study, we investigate the potential for adapting the PNA process to RAS relevant conditions with low TAN concentrations, to seek out an economical atlernative to solve the nutrient discharging problem of RAS. A continuous PNA moving-bed bioreactor (MBBR, 14L) was started up by applying active AnitaMox carriers, which originated from wastewater treatment plant treating wastewater with approximate 1000 mg TAN and 3000 mg COD per liter. The PNA-MBBR was fed with synthetic water (10 mg/L TAN and 15 g/L COD,) with a hydraulic retention time of 2 days at 20□. The PNA-MBBR showed an efficient nitrogen removal ability after around 4 weeks adaption. The TAN removal rate gradually increased from around 40% (week 5-week 10) to around 75% (week16-week 23) . After decreasing the inlet TAN to 5mg/l, the TAN removal rate has been stable around 90% for 6 weeks with an outlet concentration of TAN at below 0.1mgl/L (0.08±0.03mg/L). No nitrite accumulation was observed during the whole trial. This experiment demonstrated that the PNA process can be applied for removal of nitrogen at RAS relevant TAN concentrations. Bacterial and archaeal biofilm microbiomes of bio-carriers will be characterized by high-throughput DNA sequencing of 16S rDNA amplicons, and the results will be presented at Aquaculture Europe 2022.

UTILISING SALMOSIM, AN IN-VITRO SIMULATOR OF THE GUT OF ATLANTIC SALMON *Salmo Salar* TO ASSESS A MANNAN PREBIOTIC FOR FERMENTATION INTO VOLATILE FATTY ACIDS BY THE SALMON MICROBIOME

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Introduction

The aquaculture industry is rapidly expanding and is challenged with improving the gastro-intestinal health of farmed salmon in the face of disease outbreaks and issues arising from novel aquafeed formulations. Prebiotics are non-digestible food additives that improve gut function and overall health by stimulating the growth of commensal microbial species. Mannan oligosaccharide (MOS) prebiotics are known to improve the gut morphology of rainbow trout by modulating microbial communities[1]respectively. The control treatment consisted of a standard commercial diet, and the MOS treatment consisted of the control diet supplemented with 0.2% MOS. Morphology of the anterior and the posterior intestine was examined with light and electron microscopy. Light microscopy demonstrated increased gut absorptive surface area in the subadult MOS group. Additionally, electron microscopy revealed an increase in microvilli length and density in the subadult MOS group compared with the control (P < 0.05. Volatile fatty acids (VFAs), the end products of anaerobic fermentation of non-digestible fiber, are known to exert multiple functional benefits on the host such as maintenance of intestinal barrier integrity[2]. Recently, we presented an *in-vitro* gastrointestinal tract model of the farmed Atlantic salmon called SalmoSim[3]welfare, and the environmental sustainability of the aquaculture industry. There is still a lack of knowledge surrounding the importance and functionality of the teleost gut microbiome in fish nutrition. In vitro gut model systems might prove a valuable tool to study the effect of feed, and additives, on the host's microbial communities. Several in vitro gut models targeted at monogastric vertebrates are now in operation. Here, we report the development of an Atlantic salmon gut model, SalmoSim, to simulate three gut compartments (stomach, pyloric caecum, and midgut. We demonstrated that the major genera from the salmon gut can be maintained within *in-vitro*, and the microbial community composition and nutritional biochemical products proliferate in SalmoSim as they do in real fish when faced with a changes in diet[3]welfare, and the environmental sustainability of the aquaculture industry. There is still a lack of knowledge surrounding the importance and functionality of the teleost gut microbiome in fish nutrition. In vitro gut model systems might prove a valuable tool to study the effect of feed, and additives, on the host's microbial communities. Several in vitro gut models targeted at monogastric vertebrates are now in operation. Here, we report the development of an Atlantic salmon gut model, SalmoSim, to simulate three gut compartments (stomach, pyloric caecum, and midgut. Here we deploy SalmoSim to assay BioMOS (a commercially available prebiotic) for production of VFAs by anaerobic fermentation.

Method and materials

SalmoSim is comprised of bioreactors where physical and biochemical parameters (pH, oxygen levels, temperature, peristaltic flow rate and addition of biofluids) are maintained to ensure similarity with the gut compartments of real salmon[3]welfare, and the environmental sustainability of the aquaculture industry. There is still a lack of knowledge surrounding the importance and functionality of the teleost gut microbiome in fish nutrition. In vitro gut model systems might prove a valuable tool to study the effect of feed, and additives, on the host's microbial communities. Several in vitro gut models targeted at monogastric vertebrates are now in operation. Here, we report the development of an Atlantic salmon gut model, SalmoSim, to simulate three gut compartments (stomach, pyloric caecum, and midgut. Here, the microbiomes of pyloric caeca of adult farmed Atlantic salmon were sampled and pre-grown for 20 days to establish stable communities. A sterilized solution of ground fish meal was pumped through a bioreactor simulating the stomach then pumped into three bioreactors simulating the pyloric caeca. The pre-grown microbial communities of three salmon were inoculated into the pyloric caeca bioreactors and subjected to an static growth period of four days, followed by continuous flow pre-treatment phase of 5 days, then a 20 day BioMOS treatment phase, and finally a wash out phase of 6 days (Fig 1). Samples were taken at three time points during each phase (nine sampling time points in total).

Thirty milliliters of digest culture was taken and centrifuged at 5000 rpm for 10 minutes. The supernatant was filtered through the Costar SpinX centrifugation tube filter and VFAs were analysed by gas chromatography at MS-Omics (Denmark). A linear mixed effect model was used to determine if VFA concentrations were statistically different between the phases. The pelleted material was subjected to DNA extraction and next-generation sequencing as described previously[3]welfare, and the environmental sustainability of the aquaculture industry. There is still a lack of knowledge surrounding the importance and

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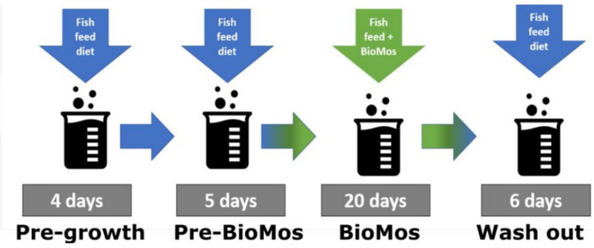

Figure 1. Visual representation of the phases of MOS prebiotic inclusion in fermentation.

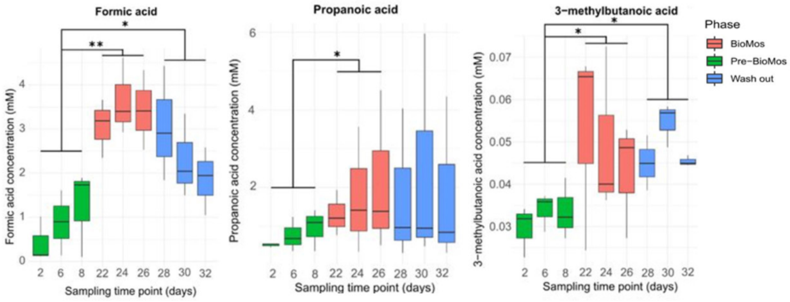

Figure 2. VFA response in SalmoSim pyloric caeca to MOS addition and washout

functionality of the teleost gut microbiome in fish nutrition. In vitro gut model systems might prove a valuable tool to study the effect of feed, and additives, on the host's microbial communities. Several in vitro gut models targeted at monogastric vertebrates are now in operation. Here, we report the development of an Atlantic salmon gut model, SalmoSim, to simulate three gut compartments (stomach, pyloric caecum, and midgut using Illumina HiSeq amplicon sequencing of the 16S V1 rDNA locus. Pearson correlation coefficients between the operational taxonomic units and concentrations of VFAs were calculated.

Results

Inclusion of the MOS increased the concentrations of the VFAs formic acid (p=0.001), propionic acid (p=0.037) and 3-methylbutanoic acid (p=0.024) whose levels correlated with increasing abundances of principally anaerobic microbial genera (*Fusobacteria, Agarivorans, Pseudoalteromonas, Myroides*). Concentrations of the VFAs 2-methyl-propanoic acid, butanoic acid, sulfurous acid, pentanoic acid, 4-methyl-pentanoic acid, hexanoic acid and heptanoic acid were unchanged by the inclusion of MOS.

Discussion

Here we assess a commercially available MOS prebiotic's effect on the production of nutritionally important VFAs by microbial flora native to farmed Atlantic salmon pyloric caeca. Interestingly, we found the MOS stimulated the production of formic acid, salts of which are often supplemented into the diets of farmed pigs to promote growth and protect from pathogens[4]. Dietary sodium propionate has also been shown to increase weight gain, increase carcass protein content and modulate immune function in European seabass[5]. Our results indicate BioMOS is a credible candidate prebiotic capable of promoting the synthesis of beneficial VFAs via the farmed salmon's native gut flora and our NGS sequencing results select genera of bacteria associated with increases in VFA production.

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THE CAUSES OF STURGEON POPULATION DECLINE, IN LOWER DANUBE

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Introduction

The special importance of Danube River is given by the fact that it represents the habitat for 6 of 26 sturgeon species: Beluga Sturgeon (Huso huso), Russian Sturgeon (*A gueldenstaedtii*), Starry Sturgeon (*A. stellatus*) and European Sturgeon (*A. sturio*), Sterlet sturgeon (*Acipenser ruthenus)* and Ship Sturgeon (*A. nudiventris*). All these species are critically endangered or some of them even probably extinct from Danube and Black Sea. Worldwide, the captures of sturgeon had the same drastic decreasing trend in the last century, as in Romania. In 1978 the fished quantity worldwide reached at 32 thousand tonnes and in 2016 decreased to only 2.2 thousand tonnes (FAO, 2018; Ionescu, 2019).

Materials and methods

The data related to the sturgeons commercial catches of are very few, we found data in old books, difficult to obtain and in database archives that are not available online. We studied and analyzed in 3 years all the specific literary sources but also the fishery databases and we found valuable data about Romanian sturgeon fishery, which have scientifically processed and interpreted, resulting very pertinent conclusions.

Results

In the seventeenth century, near Silistra (Danube, km 375), every day were caught 70 big beluga, (Decei, 1976) and, in comparation, in the year 2004, in all Romania, were commercial caught only 100 specimens. Analysing the commercial catches data, we can conclude that after a century, the Russian sturgeon commercial catches decreased with 99.7%, from 10 570 specimens (145 000 kg) in 1903–1904 to only 28 specimens (555 kg) in 2003–2004. In the case of the Beluga sturgeon, the number decreased with 97%, from 4 254 specimens (230 000 kg) in 1903–1904 to just 111 specimens (11060 kg) in 2003–2004 (Antipa, 1909; Manea, 1980; MPADR, 2006; Ionescu, 2019). The situation is also confirmed by caviar production, quantity decreased from 17 tonnes/year to 3.4 tonnes exported/average/year (Daia, 1926; FAO, 2018; Ionescu, 2019).

The Danube sturgeon population was affected by many factors like: overfishing, habitat loss, poaching, spawning site alteration, pollution, dikes, chemical changes of Danube stream water, alien species etc. We analysed the first three causes because we consider them to be the most important, with clear evidence.

Sturgeons commercial fishing, that was practiced in the last centuries without a sustainable vision, directly affected the sturgeon spawners and subadults. This was possible because of the legislation gap, due to which this activity turned into overfishing. As an example, from 1896 to 1974, the Romanian legislation stipulated too small minimum catch size of sturgeons.

For Beluga sturgeon the minimum catch size, regulated by law from 1896, was 100 cm (approx. 5–6-year-old) standard length and in 1974 increased to 170 cm. Analysing commercial catch data, it appears that before 1974, for 80 years, more than 80% of beluga caught commercially, were sub-adults (Beluga sturgeon reaches sexual maturity at an average age of 15 years). For Russian sturgeon, the minimum catch size, regulated by law from 1896, was 80 cm (approx. 6-year-old), after 80 years increased to 140 cm. Analysing the same data, in the case of this species, over 50% specimens, caught commercially, were sub-adults (Russian sturgeon reaches sexual maturity at an average age of 13 years).

Reaching sexual maturity is a primary condition of the species to ensure a sustainable population and this type of overfishing has produced consequences that is difficult to recover.

Habitat loss by blocking migration corridors and destroying the spawning sites is the second major cause that has directly affected the Danube Sturgeons. On Danube River the biggest obstacle for sturgeons is the Iron Gates I (1972) and Iron Gates II (1984) dams. The construction of the IG I (Km 942) and IG II (Km 863), blocked the migration corridor, reducing the migration with 64 %.

Iron Gates it wasn't just a crossroads for sturgeons, there were the best spawning sites, which now are permanently lost (IG I dam accumulation lake). In 1726, Marsigli describes that in the Iron Gates area, 50–100 Beluga were caught every day, near Adakaleh Island (Marsigli , 1726). Antipa also described that sturgeon are mainly reproduced in the Iron Gate cataract. At the beginning of the twentieth century, in spring, fishermen caught in this area very largest specimens of sturgeons, with a lot of amounts of caviar (Antipa, 1909).

The first and only complete study of benthic fauna from Danube cataract (km 955-1042) was realised between 1958-1960 (Popescu & Prunescu, 1961). The structure of the substrate, in this sector, was composed of boulders, gravel, coarse sand, fine sand to muddy-sandy. This structure is the ideal substrate for sturgeons spawning grounds but also for hatching, pre-development and sturgeon growth in the early stages. For example, beluga fingerlings switch to active feeding at the size of 2-4 cm and in the first stage the main food consists of tubular worms. After 3-5 cm, gammarids begin to appear in the food structure after 8 cm the larvae of chironomids and fish (Leonte, 1959). The structure of the benthos was composed by tubular worms, Gammaridae and Chironomide larvae (Popescu & Prunescu, 1961). There were many spawning sites in this area but there was also a nursery for sturgeon fingerlings, before migrating downstream to the Black Sea. Loss of these sites directly affected natural productivity and is a major cause of sturgeon populations decline, which is unfortunately irreversible.

The third major cause is poaching, which continues to cause losses of Danube Sturgeons wild populations. The poaching phenomenon was encountered in Romania, especially after the fall of communism, when the chaotical transition allowed illegal fishing and trade. The poaching persisted also after 2005, when the Romanian law banned the sturgeon fishery, and it has become more complex and harder to stop. Most of those who catch sturgeons illegally are commercial fishermen, targeting other species (eg carp), but, intentionally or not, bycatch sturgeons, because the fishing gear and method are the same. It is hard to identify this activity because they have all the legal documents for commercial fishing, transformation into poachers is made by keeping the sturgeon specimen and trafficking it on the black market. There are also fishermen who fish for scientific purposes and whom, through this cover and the negligence of the authorities, manage to traffic sturgeon specimens, especially females, which normally must be monitored, tagged and released back into the wild. In recent years, 477 adult sturgeons have been monitored by scientific fishing and tagged and of them, 91% are male (M.A.D.R., 2021), in the context in which the sex ratio is aproximativ1: 1.

Discussions

Many efforts are made for sturgeon conservation, from a general ban, stoking programs, improved legislation, enforcement to awareness, but nevertheless, there are still causes which need to be resolved.

In this context, after hundreds of years of overfishing, irrational fishing, habitat destruction, poaching and other causes that have affected Danube sturgeon populations, recovery will be slow, difficult and it will require a lot of involvement.

IMPROVED HEALTH STATUS OF ATLANTIC SALMON (*SALMO SALAR*) DURING SMOLTIFICATIONS AFTER REPLACING FISH OIL WITH ALGAL OIL

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Introduction

Atlantic salmon farming is regarded one of the most profitable and successful aquaculture industries, especially for Norway, Chile and Canada (Asche et al., 2013). To keep the production cost low, salmon feed industry has gradually shifted from marine to terrestrial plant ingredients (Aas et al., 2019). However, the use of such ingredients can have negative effects on the growth performance and health status of the fish. Therefore, microorganisms and their extracted oil, are likely to be more suitable and sustainable ingredients for the near future, due to several factors such as vitamin and mineral content, good amino acid profile, sterols, folic acid, nicotinic acid, and the capability for producing omega-3 LC-PUFA fatty acids and especially DHA (Taelman et al., 2013, Kousoulaki et al., 2013). This fatty acid is crucial for the growth, physiology and disease resistance for both fish and humans.

Materials and Methods

The trial was performed at LetSea, Dønna Norway, with the aim to evaluate the potential of *Schizochytrium* oil (*Veramaris* - VMO), partially or total replacing the fish oil. After the acclimation period, fish were randomly distributed to 9 fiberglass tanks, 200 fish/tank. There were three different dietary treatments, in triplicate, with three levels of *Veramaris,* 0% VMO, 50% VMO and 100% VMO. After 49 days of feeding, weights were recorded and various samples were collected and preserved for further analysis, such as histomorphometry of the intestines and gills, expression selected genes in the gills, and assessment of the welfare status. Fish were also assessed for saltwater tolerance in 33 ppm saltwater after the feeding period.

Results

The results have so far showed that fish fed the 50% VMO diet tended to have higher growth performance. No fish mortality was observed in fish moved to salt water. Algal oil appeared to improve the height of the intestinal folds, both in distal and anterior part, although no significant changes were observed among the treatments. Gill morphology, and gene expression involved in immunity are underway.

Conclusions

The present study has revealed so far that using of algal oil can improve the growth of fish and intestinal fold height in Atlantic salmon. Further analysis of gill morphology and gene expression involved in immunity will help to explore the physiological functions and immune responses during smoltifications when algal oil is used in the diet.

Acknowledgements

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THE IMPORTANCE OF CHOOSING THE RIGHT ORGANIC ACIDS BLEND FOR AQUACULTURE FEED

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The quest for the best performing feed additive is a major mission in the aqua feed world. There is a variety of useful additives that raise the crucial debate regarding the selection of the best option. Acidifiers are functional feed additives with acidic properties. There are many organic acids available to the animal feed industry, including formic acid, acetic acid, propionic acid, lactic acid, fumaric acids, citric acid, sodium formate, butyric acids, sorbic acids and malic acids and the question is: how to choose the appropriate and most beneficial acid blend. Many factors need to be taken under consideration such as technological and nutritional aspects to achieve a unique blend that will able to be incorporated in aqua feed and offer the best solution.

FEEDING SEABREAM IN SUBSTITUING FISHMEAL AND FISH OIL BY FRESH MUSSEL AND FEED BASED ON VEGETAL RESOURCES

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Introduction

Feeding seabream (*Sparus aurata*) on a sustainable fish feed implies to find alternatives to the use of fish oil and fishmeal in the diet. It means that attention must be paid to substitute long chain poly-unsaturated fatty acids (LC-PUFA) and essential amino acids from fish oil and fishmeal, with other sources, in order to maintain growth performances. Seabream is a predator for shellfish and is able to crush the shells, once the size is suitable. The area of Charente Maritime (France), is known to produce oysters and mussels. A part of the production, out of calibration, is discarded whereas it could be a suitable source of nutrient for gilthead seabream. Thus, to propose a sustainable diet, based on local resources, a formulated feed based on vegetal resources, supplemented with fresh mussels (*Mytilus edulis*) out of calibration was tested. In order to test the interest of this approach, two trials were carried out in the facilities of the Lycée de la Mer at Bourcefranc-le-Chapus (France): (i) An indoor experiment was conducted on small size gilthead seabreams in order to check the relevance of the experimental diet on rapid growth rate individuals without any other potential natural sources of nutrient; (ii) An outside second experiment was performed in which the experimental feed was delivered to larger seabreams in fishpond.

Materials and methods

Indoor experiment

The experiment lasted for seven weeks. The gilthead seabream were bought in a commercial hatchery at a mean weight of 6.7 g (± 1.3 g). At the beginning of the experiment, they were stocked at a density of 1.5 kg/m^3 in 3 tanks of 1.6 m^3 (342 to 345 fish per tank). The three tanks were associated in a single recirculating aquaculture system (RAS). The water circulated by air-lift and was maintained to a constant temperature of 21±1°C. Water was renewed at a rate of 2 m^{3}water per kg of feed distributed. In each rearing tank, a different diet was delivered 6 days a week: (i) the "control" diet (C), corresponding to a commercial feed (Neo Start, Le Gouessant), specific to pre-growing marine fish (including marine ingredients); (ii) the "vegetable" diet (V), corresponding to an experimental formulated feed composed only of raw materials from plants. This experimental feed was formulated based on: (1) absence of marine ingredients; (2) use of raw materials only from European production; (3) without synthetic amino acids; (4) limitation in the use of refined products; (5) a protein/fat rate similar to the commercial feed; (6) and use of an attractive compounds to foster feed intake (Actipal HP1-S1. Dianaaqua); (iii) the "mussel" diet (M) corresponding to the formulated feed V (five days a week), supplemented with fresh flesh (shells were removed) from discarded mussels, on the basis of gross energy content one day a week. The mussels were purchased from a close aquaculture farm.

Outdoor Experiment

The outdoor trial was performed twice in 2020 and 2021, from early June to early October. The gilthead seabream were bought in a commercial farm, from an indoor controlled environment. At the beginning of the experiments, the fish had a mean weight of 200 g and were stocked at a density of 3 individuals/m^2 in a single earthen pond of 410 m^2. The fish feed was formulated on the same basis than in the previous experiment and adapted to the requirement of 200g seabreams. The formulated feed was delivered five days a week, supplemented with discarded whole fresh mussels on the basis of gross energy content one day a week.

For indoor and outdoor experiments, fish were weighed at the beginning and the end of the experiment, and at intermediate dates. During the experiments, the quantities of feed distributed were recorded. In each experiment, proximate composition of diets, mussels and whole body were analyzed and their fatty acid profile determined, at the beginning and at the end of the experiment.

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Results

Indoor experiment

At the end of the experiment, the mean weight of fish from the C (23.1 ± 4.3 g) and M (23.1 ± 5.2 g) treatments were not significantly different, whereas they were significantly lower in the V treatment (15.5 ± 3.6 g) ($p<0.05$). The survival rate was slightly higher in C and M (99.7% and 99.4% respectively) than in V (98.0%). Feed Conversion Ratio (FCR) obtained for C, V and M were 1.00, 1.84 and 0.99, respectively. For M, The FCR, including the mussel, calculated on the basis of the formulated feed at 97.3% dry matter (DM).

A total of 5 182 g DM of feed were delivered in C, 5 057 g DM in V and 5 240 g DM in M (composed of 4 708.5 g DM of feed V and 531.5 g DM of mussel flesh). The quantities of total protein delivered in each treatment were 2 891 g for C, 2 781 g for V and 2 906 g for M, and the quantities of total energy delivered were 110 MJ for C, 122 MJ for V and 116 MJ for M. During the experiment, the lipid content of the fish increased, with a higher level in V and M (11.87% and 12.15% respectively) than in C (9.38%), at the end of the experiment. But, the proportion of EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) in the body lipids was lower in V (1.31% and 2.10%) and M (1.82% and 2.21%) than in C (9.20% and 10.78%, respectively), at the end of the experiment. The retention rates in lipids and protein were similar between C (69% and 30% respectively) and M (70% and 29% respectively) but lower for V (45% and 16% respectively).

Outdoor Experiment

Similar growth performances were observed between the trials carried out in 2020 and in 2021 with a final mean weight of 352.4 g (± 59.4 g) and 363.8 g (± 72.1 g) respectively. The survival rate was similar too with 94% and 95% respectively. The FCR obtained were 1.90 and 1.76 respectively, calculated on the basis of mussel with the same dry matter than the formulated feed. During the experiment, the mean lipid content of the fish increased, from 13.8% to 17.5%, but, the mean proportion of EPA and DHA in the body lipids decreased from 3.9% to 1.8% and from 7.1% to 3.5% respectively. The retention rates in lipids and protein were 16% and 18% respectively.

Discussion-Conclusion

For both types of experiment, fish fed with formulated feed supplemented with fresh mussel exhibited growth performances similar to that from fish fed with commercial feed. Survival rates observed were over 90%.

If the protein content in fish remained stable during the experiments, the lipid content increased. The balances between saturated and polyunsaturated fatty acid, and n-3/n-6 FA ratio decreased with the use of the vegetable diet, and the supplementation with mussel did not allow to maintain the proportions of PUFA.

The use of the proposed feeding strategy has to be confirmed on longer periods and/or included in a global feeding strategy along the fish rearing cycle, including phases of feeds of different PUFA levels. This study shows that it is possible to propose new feeding strategies, based on the use of available nutritive sources in territories.

CARRYING CAPACITY AND SPATIAL VARIABILITY OF THE CULTIVATED *Saccharina latissima* IN A DUTCH COASTAL BAY: A MODELING ASSESSMENT

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Introduction

Kelp cultivation receives increasing interest for its high-value products and ecological services, especially in Europe and North America. Before industrial kelp farming in marine ecosystems continue to scale up, evaluation of the site-wide production relative to ecological carrying capacity (CC) of the identified system is essential (Jiang et al 2022). Furthermore, prior studies have demonstrated large spatial variability in the yield and chemical composition of the cultivated *S. latissima*, even within small coastal bays. Hence main drivers of the spatial variability must be investigated to offer insight into farm-site selection and optimize production.

Methods

For the purpose of evaluating ecological and production carrying capacity, a mechanistic kelp model was developed and applied for hypothetical numerical experiments of expanding the farming area in a Dutch coastal bay (the Eastern Scheldt, Figure 1), where cultivation of *Saccharina latissima* (sugar kelp) is emerging. The kelp model was implemented within a three-dimensional hydrodynamic–biogeochemical model to account for the environmental interactions. The model captured the seasonal growth dynamics of *S. latissima*, as well as its carbon and nitrogen contents measured at the Eastern Scheldt pilot sites. A total of two types of scenarios were run in this study: the baseline scenario representing the current pilot sites and idealized scenarios increasing the farming area hypothetically by 1%, 3%, 10% and 30% of the total bay area, respectively, where farm locations were uniformly distributed not considering any potential (physical) conflict with other user functions.

Using the same a pre-validated hydrodynamic-biogeochemical-kelp model, we examined the spatial variability and investigated the main drivers in *S. latissima* growth dynamics in 40 hypothetical farms spread throughout the bay. Meanwhile, the Eastern Scheldt, renowned for mussel and oyster culture for decades, serves appropriately as a prototype for studying the reciprocal effects between (co-cultivation of) shellfish and farmed *S. latissima*.

Results & Discussion

The model results suggest that expanding the kelp farming area to ~1–30% of the bay (representing ~3.4–75 kt harvest dry weight in the 350-km2 bay) had the potential to weaken the spring bloom (Figure 2), and thereby affected the coexisting shellfish culture in the bay. Competition between *S. latissima* and phytoplankton mostly occurred in late spring for nutrients (dissolved inorganic nitrogen). The ecological CC should be weighed according to these negative impacts. However, the production CC was not reached even when farming ~30% of the Eastern Scheldt, i.e. harvesting totally 75 kt dry mass, given that the simulated overall *S. latissima* production kept increasing with the farming activity.

S. latissima yield in the deeper and better flushed western compartment more than doubles that in the eastern compartment, which is found to be driven primarily by temperature. Model results furthermore suggest that *S. latissima* benefits from co-cultivation with shellfish, since nutrients excreted by shellfish replenish its nitrogen reserve and fuels a relatively high growth rate in the nitrogen-depleted late spring.

Our modelling approach can be applied to other systems for *S. latissima* cultivation and assist in assessing CC and environmental impacts. We also discuss the possible further improvements for assisting in seaweed farming practice.

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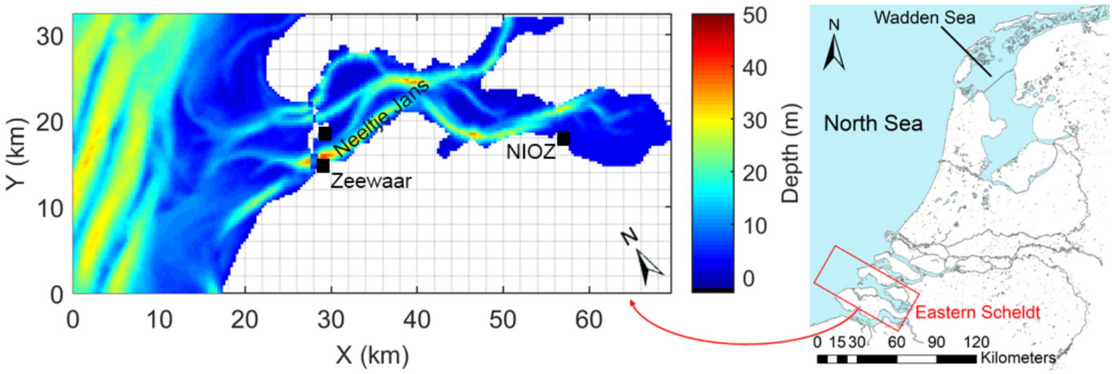

Figure 1. The location of the Eastern Scheldt (the right panel) and the model domain (the left panel). The Eastern Scheldt is divided into four (western, central, northern, and eastern) compartments, as separated by black lines. Sites 1 and 2 in the left panel indicate two commercial farms providing field samples for this study (Source: Jiang et al 2022)

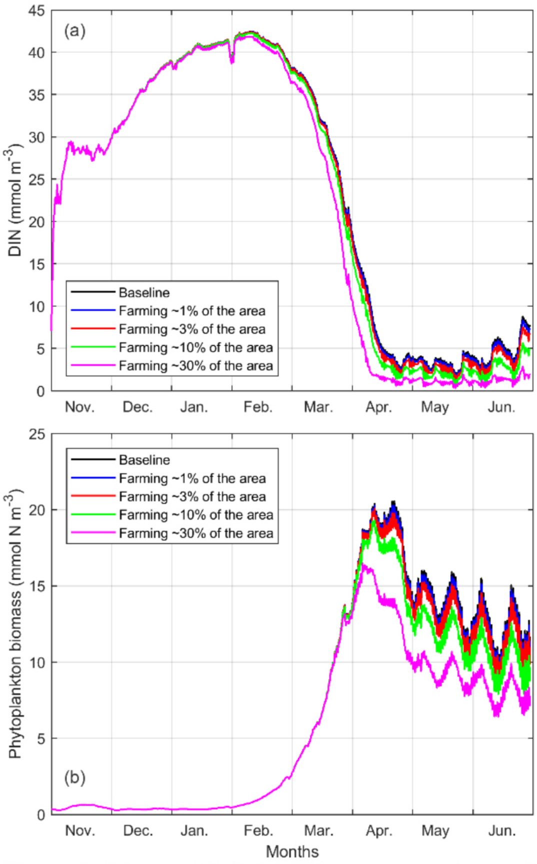

Figure 2. The modeled (a) DIN concentration and (b) phytoplankton biomass from November 2009 to June 2010 in scenarios varying farming areas (farming 1%,3%,10%, 30% of the area of the bay) (Source: Jiang et al 2022)

BY-PRODUCT FROM COMMERCIAL PRODUCTION OF THE DIATOM *Phaeodactylum tricornutum* FOR THE DEVELOPMENT OF ALTERNATIVE TREATMENT AGAINST PARASITIC DISEASES IN AQUACULTURE

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Introduction:

Parasites are ubiquitous in aquaculture and can have devastating effect with serious socioeconomic, ecological, and welfare consequences. Of these, monogenean parasites are of particular problem as the conventional treatment for this infection, application of organophosphates is no longer permitted due to its neurotoxic effect, leaving no treatment option for this disease, which induces high morbidity and mortality due to skin and gill lesions associated with the attachment of the parasite to the fish.

In the past years there is a trend in aquaculture of moving away from chemical to natural treatments, *i.e.* alternative strategies for disease control and natural treatments effective in eradicating fish parasites and safer to public health and the environment.

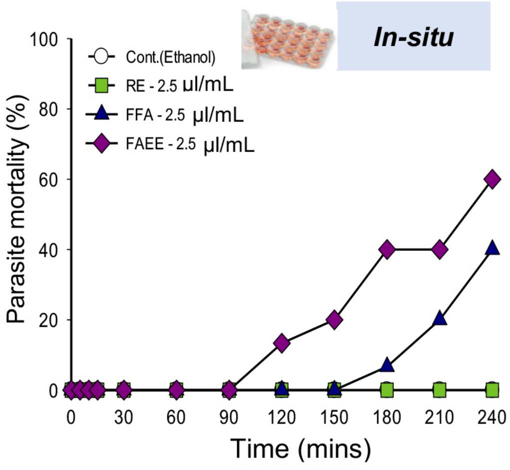
Our previous research had demonstrated that an extract produced from the diatom *Phaeodactylum tricornutum* was effective against fish monogenean parasites. *P. tricornutum* is a potent photosynthetic marine diatom, which is commercially produced for high value nutraceuticals, such as LC-PUFA and fucoxanthin. Its extracts also have been shown to have antibacterial properties, mainly associated with fatty acids liberated from glycerolipids during cell treatment and extraction.

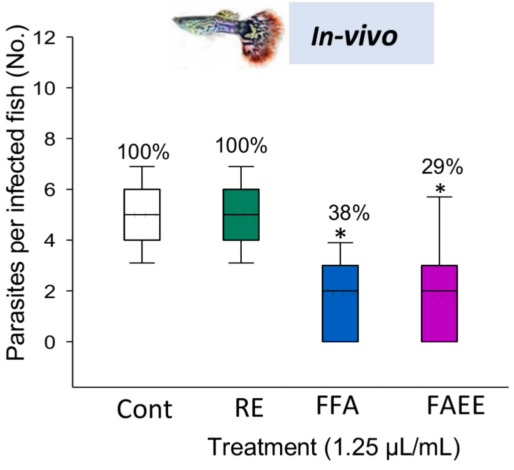
To further develop a sustainable and cost-effective treatment for aquaculture, the use of residue material from commercial *P. tricornutum* production was explored.

Methods:

Gyrodactylus turnbulli, a monogenean parasite affecting guppies (*Poecilia reticulate*) was used. As infection is high on the guppies' tail fin which is transparent, direct observation of live fish and tail clips is feasible. Residue material of *P. tricornutum*, which was produced as a source of fucoxanthin, was obtained as dry powder from a commercial producer. Several extracts and preparations were tested, including Ethanolic residue extract (RE), free fatty acids (FFA) produced from this residue extract (RE) and fatty acid ethyl esters (FAEEs), produced directly from the *P. tricornutum* residue powder. These preparations were initially tested *in situ*, using infected tail clips, followed by *in vivo* testing.

To characterize the active ingredients, the extracts and preparations were analyzed by thin- layer chromatography (TLC), GC, followed by direct examination of pure compounds with potential anti-parasitic toxicity that were identified by these analyses.





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Results:
All extracts and preparations were effective against the parasite, but FAEE fraction was the most effective *in situ* and *in vivo* (Fig 1). FAEE can be easily produced by a single step, direct transethylation of the algal residue powder. The active ingredients included FAEE of EPA 16:0, 16:1 and 14:0.

The research presents the potential utilization of *P. tricornutum* residue as a source for natural treatment against *G. turnbulli* and potentially other monogenean parasites.

INFLUENCE OF TEMPERATURE AND DIET QUALITY ON LARVAL PERFORMANCE OF *Palaemon serratus*

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Introduction

The pink shrimp, *Palaemon serratus*, is a commercially valuable species distributed along the European coast. It lives for 2-5 years and females can weigh up to 13 grams (Huxley, 2011). Although aquaculture trials were attempted in the 1970s and 1980s (Campillo, 1979; Rodriguez, 1981), this species is not commercially produced today. Due to its size and the temperature of the environment in which it naturally lives, *P. serratus* is an ideal candidate for extensive European marine shrimp farming.

Here we focus on the performance of the larval phase of *P. serratus* under controlled conditions. Temperature determines the duration of larval development by influencing the number of larval moults and the duration of intermoult (Gonzalez-Ortegon and Giménez, 2014). This temperature-mediated plasticity has consequences for juveniles, with size at metamorphosis decreasing with increasing temperature (Baudet et al., in press). Conversely, larval survival increases with temperature (Reeve, 1969). The rate of metamorphosis of *P. serratus* larvae is very low at temperatures below 15°C (Reeve, 1969). However, the larvae of this species are released into the environment between April and July, in areas where the water can be between 10 and 15°C. We believe that the poor larval performance obtained in the laboratory in cold water is due to the poor diet used. Indeed, artemia nauplii contain little of the polyunsaturated fatty acids necessary for membrane fluidity in cold water (Sorgeloos et al., 2001).

In this study, we compared the larval performance of *P. serratus* at three temperatures (14, 18 and 22°C) by feeding the larvae three diets of more or less rich artemia. We hypothesise that larval survival increases with diet richness and temperature. The interaction of these two factors should have synergistic effects on larval survival. We also expect that diet richness will increase growth rate, regardless of temperature.

Materials and methods

We collected 5 ovigerous *P. serratus* females which we isolated in the laboratory. Upon hatching of each brood, 9 groups of 52 larvae were incubated in 9 conical vessels of 500 mL. These were then incubated in 3 waterbaths at 14, 18 and 22°C respectively (3 vessels of each brood per waterbath).

To test the influence of the fatty acid richness of the diet on larval development, we prepared the following 3 diets (from the poorest to the richest): unenriched metanauplii I (24h after hatching), nauplii I (at hatching) and metanauplii I enriched for 24h with microalgae rich in polyunsaturated fatty acids. Each vessel was fed the same diet every day, *i.e*. 10 artemia per mL. We therefore had 45 containers each containing 52 larvae (5 females x 3 temperatures x 3 diets). The larvae were observed during the water change 3 times a week. When they metamorphosed into juveniles (morphological and behavioural criteria), they were removed for weighing and stored individually at -80°C. Their lipid profile was then analysed.

We will test the effects of temperature and diets on larval metamorphosis rate, development time and weight of metamorphosed larvae. For this purpose, we will use mixed-effects models to consider that for each model, the larvae used at different temperatures were from the same females.

Results and Discussion

The experiment started in May 2022 and is currently not completed. The analysis of these and the discussion will be presented at the conference.

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IMPROVING THE VITALITY OF EUROPEAN EEL LARVAE

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Introduction

Because the life cycle of the European eel *Anguilla anguilla* is not yet closed in captivity, the aquaculture industry depends on wild caught juvenile glass eels that are grown to marketable size. In our facilities, larvae are produced on a regular basis but they die without feeding exogenously. High mortality and deformity rates are often observed during the first week after hatching. Therefore, there is an urgent need to increase egg and larvae quality to produce larvae that can grow, survive and metamorphose into glass eels. In an earlier study (Jéhannet et al., 2021), we compared the transcriptome of larvae at 1 dph, of batches that survived less than 3 days post hatch (dph) *vs*. batches that survived for at least a week to understand the reasons behind the mortality rates during the first week after hatching. Our results suggested that larvae that die early suffered from microbial infections because the expression of genes related to inflammation and host protection were up-regulated when compared to gene expression in viable larvae. Therefore, in this study, we tested the effects of antibiotics (rifampicin and ampicillin 50 mg.l^{-1}) and egg surface disinfection treatment (povidone iodine 25 ppm), alone or in combination, on hatching success, larvae survival and deformities in larvae from wild and feminized eels.

Materials and methods

Eggs were stripped from wild eels (N=5) and from eels that were feminized during the young elver stage (N=12). Eggs were collected in dry bowls and each individual batch was mixed with the sperm of 6-8 males. Gametes were activated by adding seawater and placed in 5-L beaker containing artificial seawater kept at a water temperature of 18°C and a salinity of 36 ppt under dark conditions. Within 2 hours post fertilization (hpf), floating eggs were collected from the 5-L beaker with a sieve and weighed. From the weighed eggs, 63 g of eggs were equally distributed over twenty-one 1,800 mL beakers containing the various treatments (Table 1). Eggs were (i) placed in three beakers containing artificial seawater as control (C); (ii) placed in three beakers containing artificial seawater supplemented with antibiotics (A2); (iii) disinfected for 5 mins, rinsed three times and placed in three beakers containing artificial seawater (D2); (iv) disinfected, rinsed and placed in three beakers containing artificial seawater supplemented with antibiotics (AD2) and (v) placed in nine beakers containing artificial seawater until similar treatments but at10 hpf. At 10 hpf, eggs that were previously kept under control conditions were (i) placed in three beakers containing artificial seawater supplemented with antibiotics (A10), (ii) disinfected, rinsed and placed in three beakers containing artificial seawater (D10); (iii) disinfected, rinsed and placed in three beakers containing artificial seawater supplemented with antibiotics (AD10). When females gave less than 63 g of eggs, treatments were applied in the priority order as shown in Table 1. For each treatment, hatching success, larvae survival and the type of deformities observed were recorded.

Results and discussion

Of the twelve feminized eels that were stripped, five of them gave larvae that survived up to 5 dph. All five wild eels that were stripped gave larvae that survived until 21 dph. In the controls for treatment, eggs from the wild eels had significantly higher hatching rates (8 %) than the eggs from the feminized females (1 %). In the controls, larvae from the wild females significantly survived longer (4 ± 1 dph) than the ones from the feminized females (1 ± 2 dph). These results show that wild females produced eggs and larvae of higher quality than the feminized ones. Antibiotic treatment doubled the larval survival period but the effect was only significant *vs*. eggs that were disinfected, not *vs*. the controls. As variations were high, we are currently increasing the number of observations. Among the abnormal larvae from feminized and wild eels, larvae were often curved. Larvae that hatched later than 80 hpf were more curved than larvae that hatch before 60 hpf suggesting that curvature is related to delayed hatching. Also other abnormalities such as notochord deformities (Fig. 1), pericardial oedema and head emaciation were frequently observed. Although our results show that antibiotics increase the early larval survival in European eels, sustainable methods aiming for antimicrobial control and increasing larvae survival are being developed.

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Table 1: Overview of the treatments used for incubation of European eel eggs and larvae.

Treatment	**Treatment composition**
C: control	artificial seawater
A2: antibiotics at 2 hpf	rifampicin and ampicillin 50 mg.l^{-1}
D2: disinfection at 2 hpf	povidone iodine 25 ppm
AD2: antibiotics and disinfection at 2hpf	rifampicin and ampicillin 50 mg.l^{-1} and povidone iodine 25 ppm
A10: antibiotics at 10 hpf	rifampicin and ampicillin 50 mg.l^{-1}
D10: disinfection at 10 hpf	povidone iodine 25 ppm
AD10: antibiotics and disinfection at 2hpf	rifampicin and ampicillin 50 mg.l^{-1} and povidone iodine 25 ppm

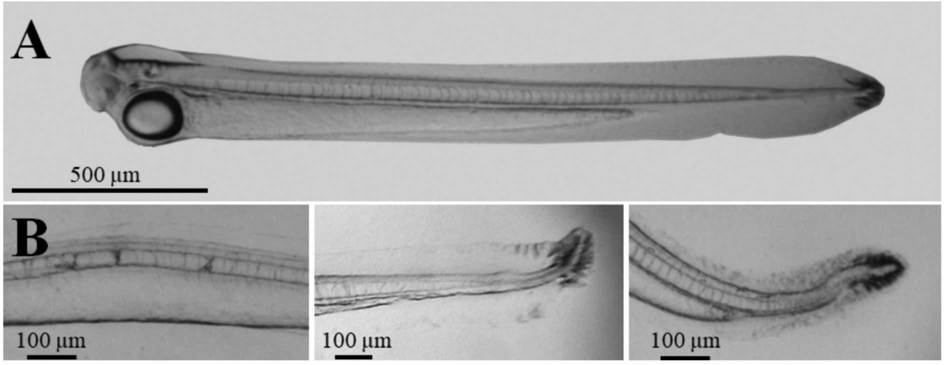

Fig 1. Larvae at 100 hpf. **A**: normal larva and **B**: various notochord deformities around the middle part of the trunk and at the end of the tail.

Acknowledgements

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COLLAPSING COASTAL GOVERNABILITY – GOVERNING UNSTABLE OBJECTS IN NORWEGIAN SALMON AQUACULTURE

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Introduction

Evolving economies and technologies in the coastal space outline a situation related to governance where the ability to govern is becoming increasingly wicked. Governability, a term coined by Kooiman et al. (2008, p.3), relates to the "overall capacity [within a governing system] to make something governable". In relation to the ongoing intensification of anthropogenic marine activity and the pursuit for sustainable solutions, the need to reflect upon governability becomes highly relevant. It is thus unfortunate that current approaches to marine governance do not seem to appropriately deal with the unwanted outcomes of the ever more closely integrated socio-techno-ecological systems (Glaser & Glaeser 2014). On one hand, coastal spaces are seen as areas of opportunity, growth, and development. While on the other hand, many coastal areas remain a breeding ground for intense conflicts of interest, causing severe deterioration of marine ecosystems (Glavovic 2013).

With the theoretical lens provided by Evolutionary Governance Theory (EGT) and a poststructuralist approach, this paper investigates how innovative technology unfamiliar to the governing system feeds into the escalating wicked situation of conflicting discourses within sustainable development, and how technology thus challenges the governability. EGT has proven useful when studying emerging relations and patterns, and in this case the theory has been applied to show how and why governability is collapsing.

The paper explores the issue of collapsing governability through relating to a case in Norwegian salmon aquaculture where a type of innovative technology seeks to contribute to sustainable development. The technology in question is a dynamic aquaculture installation called Havfarm 2 (HF2). With its dynamic features, the installation will have the possibility to move around to always maintain optimum farming conditions, but also to adapt to other (industrial) activities making use of the same space. However, issues of governability arise as the governing system does not know how to govern a dynamic installation primarily because aquaculture is seen as, and regulated as, a physically stationary activity. The relevant research question, thus, becomes: "How is the governability of coastal areas challenged by innovative aquaculture technology?".

Methods and materials

The case of HF2 was chosen because it illustrates what happens when technology development reaches a point where it breaks with what the governing system is familiar with, and thus challenges the governability of the system. Following constructivist traditions, the methods of document analysis (Asdal & Reinertsen 2020) and process-tracing (George & Bennett 2005) have been made use of when analyzing gathered data. With an abductive and iterative approach, the research question has been explored by gathering data from governmental documents, reports, white papers, and news articles that relate either directly to developments concerning HF2 or salmon aquaculture development in Norway in general. The latter to situate the events relating to HF2 in a larger perspective. Retriever Atekst (Norwegian news database) and governmental websites are the databases used to find material.

Theoretical framework

EGT understands governance as the result of the evolution of many elements, such as actors, institutions, knowledge, and discourses, as well as technology. In established academic literature, EGT has proven useful to understand the specificity of coastal governance and the possibilities of a coastal governance better adapted to challenges that arise through intensified use of the sea (Van Assche et al. 2020). Through mapping governance paths and shifting roles of knowledge and narrative, the theory offers concepts that help understand how HF2 becomes an unstable object that is challenging the governability of the governing system.

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Results and Discussion

The acquired data tells the tale of what happens when technology exposes the lack of governability within a governing system, and why it happens. The governing system of Norwegian aquaculture is not constructed to handle the type of governmental dynamism necessitated by HF2 because the technological solution it represents comes across as unknown to the governing system. The evolution of actors, institutions, knowledge, and power have led to emerging governance paths over the past years that indicates a governing system that seeks to develop the industry with use of other technologies than dynamic aquaculture due to lack of governing capacity for such a technology.

The absence of suitable governing arrangements, regulations, and knowledge about what dynamic aquaculture implies has resulted in an ever-evolving web of politics, stakeholders, resistance, and sciences that is reducing the governability of the governing system. This outlines a reality where governability is collapsing in its attempt to govern an object that represents instability, i.e., unknown technological solutions.

What this case study ultimately shows is that the governing system and its institutions are integral for technology to work as intended. Even though technology, isolated, might represent a contribution to sustainable development, the technology is nothing without supportive policies and regulations. Hence, this paper argues that sustainability is not something that is achieved through technology, but that sustainability is a continuous process that is advanced through the necessary constant performativity of policies and politics.

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REGULATION OF AQUACULTURE PRODUCTION

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Since the late 1980s, global capture fisheries' production for human consumption has stagnated, whereas the output from aquaculture has increased rapidly. Today, aquaculture deliver nearly 50% of the fish used for human consumption worldwide. However, food production from aquaculture generates a number of negative externalities, including nutrient discharges, implying that management may be necessary. However, to a large extent, current governmental regulation is undertaken by using command and control instruments, with limits on water use as an example, and these instruments are considered economically inefficient. Therefore, the purpose of this paper is to study the economically optimal regulation of aquaculture production. The time between releasing and harvesting fish (rotation time) is an important decision variable for aquaculture producers, and we use rotation time as a regulatory variable. By drawing on ideas from the forest economics literature, we construct basic models for a private and social optimum, assuming an infinite time horizon. Furthermore, we explicitly introduce the quality of fish into our basic models and show that an optimal marginal tax is equal to the marginal damage costs (with respect to time) in the current and all future rotations (a dynamic Pigovian tax). We also discuss the implications for the regulatory recommendations of relaxing several assumptions in the basic models, such as the time when costs are incurred, differences between the social and private revenue functions and differences between the private and social discount rates.

FLEXIBLE POLICY INSTRUMENTS TO ENCOURAGE EXTERNALITY ABATEMENT TECHNOLOGIES IN SALMON AQUACULTURE

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Aquaculture has been identified as a food sector that holds potential to grow sustainably to provide important sources of protein and essential micronutrients to an increasing global population, while maintaining a low environmental footprint relative to other food production technologies. Simultaneously, there is a shift in society's priorities with the threat of climate change taking a much more prominent role. In Norway, the political consensus is that an increase in salmon aquaculture production from today's 1.5 million tonnes to 5 million tonnes is desired by 2050 but only if achieved in an environmentally sustainable manner. Paradoxically, this ambition comes at a time when conventional open cage salmon aquaculture operations globally have been experiencing limited growth in production due to environmental challenges.

In response to a range of negative externalities, but also driven by the need for a higher degree of control over the production environment, salmon producers in Norway have been heavily investing in new technologies that hold promise of externality abatement. These technologies range from onshore production to closed and semi-closed containment systems and offshore open production systems. Although the range of negative externalities in salmon aquaculture is broad, we focus the discussion on regulating a single externality, namely sea-lice. This choice is motivated by sea-lice being an externality central to the regulatory framework in Norway, but also by the need to maintain clarity in the discussion, as a system with several regulatory instruments can quickly become complicated. Norway, like other salmon producing nations, has designed its regulatory measures to control the spread of this parasite through command-and-control measures

Unlike command-and-control instruments which offer no incentives to address externalities beyond the standard set by a related piece of regulation, economic incentives encourage behaviour through price signals rather than through explicit instructions. Economic incentives promote investments in new technologies by directly imposing a price on the externality. A result from regulatory economics is that a tax on pollution and a subsidy to abatement ultimately lead to the same level of pollution. Investments in new technologies that reduce the sea-lice problem are by nature dynamic. We propose and discuss feed-in-tariffs as a dynamic subsidy that encourages investments in new technologies. We present lessons-learned from Germany and Spain where these tariffs have been successfully used to promote investment in renewable energy production and draw parallels to the Norwegian salmon aquaculture setting.

The choice of discussing dynamic subsidies in the context of a single externality is equivalent to accepting that other externalities in salmon aquaculture production remain unregulated and that a subsidy to sea-lice reduction may have a less desirable effect on the other externalities. In practice however, several externalities may arise simultaneously (e.g., sea lice infestation and genetic pollution). Possible regulatory solutions may be to apply feed-in-tariffs to each externality separately, prioritizing the most important externality, or using an index of several externalities.

EVALUATING TWO METHODS OF INCORPORATION OF THE HALOPHYTE *Salicornia ramosissima* IN WHITELEG SHRIMP DIETS (*Penaeus vannamei*): SURVIVAL, GROWTH AND FEED EFFICIENCY

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Introduction

To sustain the growth of the aquaculture sector, it is of paramount importance to study and develop efficient diets with alternative raw materials. Ingredients traditionally used in aquafeeds, such as wheat, are increasingly scarce and costly given the growing demand´s to supplement human and animal nutrition. The production of halophyte plants for human consumption, such as *Salicornia ramosissima,* is increasing as they have the ability to grow in saline soils or be irrigated with seawater allowing the utilization of unexploited resources. Additionally, these are known for their richness in bioactive secondary metabolites which can have health promoting effects for the consumer (e.g., phenolic compounds). However, the tender green tips of Salicornia are the only part sold as food, being essential to value the remaining plant material wasted. The principles of circular economy, where waste is recovered and utilized to create added value, are increasingly important, being applied to economical relevant areas, including the aquafeed industry. The whiteleg shrimp (*Penaeus vannamei*) is the most produced crustacean species worldwide. However, feed associated costs are significant, making the identification of more economical and sustainable formulations essential for the success of shrimp farming. Therefore, the current study aimed at evaluating the potential of incorporating *S. ramosissima* biomass in diets for juvenile *P. vannamei*. For this purpose, shrimp survival, growth and feed efficiency performances were assessed in two different experiments: Experiment 1 (dried non edible biomass) – inclusion of stems (SS) and leaves and seeds (SL) at 5% and 10% inclusion levels, replacing wheat; Experiment 2 (green protein rich extract) – supplement diets with *S. ramosissima* extracts at 0.1% and 1% inclusion levels.

Methods

Experiment 1: Five experimental diets were tested (5 replicate tanks each): a commercial like diet (Control), two experimental diets containing *S. ramosissima* stems at 5% and 10% inclusion levels, replacing wheat (SS5 and SS10, respectively), and two containing *S. ramosissima* leaves and seeds at 5% and 10% inclusion levels (SL5 and SL10, respectively). In this trial, the inclusion of *S. ramosissima* replaced Wheat in the same proportion. Whiteleg shrimp juveniles (mean wet weight 6 g) were kept at around 28 ºC and fed *ad libitum* for 55 days. At the end of the trial, shrimp were weighted and counted for growth performance and survival determination. Experiment 2: Three experimental diets were tested (6 replicate tanks each): a commercial like diet (Control), two experimental diets containing *S. ramosissima* extracts at 0.1% and 1% inclusion levels (SE 0.1% and SE 1%, respectively). Whiteleg shrimp juveniles (mean wet weight 1.6 g) were kept at around 28 ºC and fed *ad libitum* for 30 days. At the end of the trial, shrimp were weighted and counted for survival, growth and feed efficiency performances.

Table 1. *Salicornia ramosissima* biomass (Experiment 1) and extracts (Experiment 2): Initial and final weight, relative growth rate (RGR), feed conversation ratio (FCR), feed intake and survival of whiteleg shrimp juveniles fed the experimental diets for 55 and 30 days, respectively.

	Experiment 1 (Biomass)					Experiment 2 (Extracts)		
	Control	SS 5	SS 10	SL 5	SL 10	Control	SE 0.1%	SE 1%
Initial weight (g)	6.1 ± 0.0					1.6 ± 0.0		
Final weight (g)	17.6 ± 0.4	18.1 ± 0.5	18.1 ± 0.4	18.1 ± 0.4	18.3 ± 0.3	5.6 ± 0.4	5.9 ± 0.3	5.5 ± 0.3
RGR (% day-1)	2.0 ± 0.0	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	3.9 ± 0.2	4.1 ± 0.2	3.8 ± 0.2
FCR	3.1 ± 0.2^{a}	3.6 ± 0.2^{b}	3.7 ± 0.1^{b}	3.6 ± 0.2^{b}	4.0 ± 0.1^{c}	2.2 ± 0.1^{a}	2.6 ± 0.1^{b}	2.6 ± 0.1^{b}
Feed intake (% ABW d^{-1})	5.3 ± 0.3^{a}	6.2 ± 0.2^{b}	6.4 ± 0.2^{b}	6.3 ± 0.2^{b}	7.1 ± 0.2^{c}	8.8 ± 1.1^{a}	10.9 ± 0.5^{b}	$9.8 \pm 0.7^{a,b}$
Survival (%)	96.7 ± 1.5	97.1 ± 2.4	98.2 ± 2.6	96.0 ± 2.7	97.8 ± 1.5	97.7 ± 5.0	99.2 ± 1.7	98.5 ± 2.1

SS: Salicornia steams; SL: Salicornia leaves and seeds; SE: Salicornia extract. Results expressed as mean ± standard deviation (n = 5 experimental units). Different superscript letters indicate statistical differences ($P<0.05$) between treatments in a One-way ANOVA.

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Results and discussion

No statistically significant differences in growth performance and survival were observed among treatments (Table 1), suggesting that the inclusion of *S. ramosissima* biomass and extracts, at least in the forms and levels tested, did not compromise the adequacy of the diets. Nevertheless, shrimp fed diets containing Salicornia ate significantly more to achieve the same weight as those fed the Control, with this effect being more prominent in the SL10 and SE 0.1% diets (of each experiment, respectively) which produced significantly higher FCR and feed intake values than the remaining diets (Table 1).

Conclusion

Data from Experiment 1 and 2 indicate that *S. ramosissima* biomass and extracts, respectively, can be included in diets for juvenile *P. vannamei* with no detrimental effects on growth performance or survival. The inclusion of *S. ramosissima* stems in whiteleg shrimp diets (Experiment 1) seems to be preferable over leaves and seeds, which is the ideal scenario for adding value to halophyte production and potentially reduce shrimp feed formulation costs. Additionally, the valorization of a residue contributes to the principles of circular economy. The shrimp feed SE 0.1% diet (Experiment 2) exhibited the best results for final weight, RGR and survival, however without statistically differences for remaining treatments. The effect of these diets on the immune status of the species is being studied and will be presented in future works.

Acknowledgements

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UTILIZATION OF SEASONAL FISH IN THE FORMULATION AND DEVELOPMENT OF FISH PRODUCTS TO IMPROVE FISH CONSUMPTION AND INCREASE THE UTILIZATION OF FISH BIOMASS

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Introduction

Fish are rich in highly digestible proteins, vitamins, minerals, omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Jia et al., 2022). Those are essential nutrients for the human body and are important to improve the daily human diet. However, fish consumption is very low in the Czech republic because of high prices, complex preparation, and food prejudices (Voráčováet al., 2018). Processing of fish is also very low (about 11%), and most fish in the market are sold alive, chilled, or frozen carcasses and fillets. There is a low diversity of fish products. The sale of freshwater fish produced in the Czech Republic is also very seasonal, and most fish is consumed during Christmas. Thus, the consumer is not very motivated to buy freshwater fish, given the laboriousness of their processing, the presence of bones, and the relatively narrow range of available fish products. This study aims to develop new fish products and fish recipes to increase the diversity of fish products and increase fish consumption. Furthermore, the development of fish products and recipes might also change human eating habits to improve health and increase the utilization of fish biomass to avoid losses.

Materials and methods

The local professional chefs designed six new fish products and 11 fish recipes for fish dishes in our processing plant. During the product and recipe development, non-/fillet parts of the fish body were preferentially used to utilize better the fish biomass, such as fish fillets, filleting skeletons, and fishtails. The new products and recipes were analyzed for nutritional composition and microbial stability, and then they were presented to the consumers and had a sensory evaluation in the restaurant.

Results

All the newly developed fish products and recipes are shown in Fig.1. According to nutritional analyses, almost all fish products and recipes were rich in protein and fat content (Table.1). Especially, P2, R5, R7, and R9 had significantly higher DHA+EHA content (>100 mg/100 g) than other products and recipes; and the ratio of n-6/n-3 is higher than 4 in P4, P5, R1, R2, and R10. Hence, almost all products and recipes displayed excellent lipid quality, which has good advantages for human health, excluding R10 because its salt content was more than the limited standards (1.7g/100g), 1.9 g/100g.

Apart from nutritional evaluation, the quality and safety are very important to be monitored during the storage; furthermore, stability is regarded as the main criterion for product selection during storage. Hence, fish products' microorganisms, TBARS, and peroxide values were analyzed. The results presented that almost all fish products had good stability during the storage for three months, excluding the smoked carp with lard because of the mold, as shown in Fig.2. Moreover, the other five new products were recommended and applied finally.

On the other hand, sensory evaluation is crucial to presenting delicious food to consumers. Here, the main criterion for selecting sensory evaluation was the overall impression criterion in sensory evaluation, with a minimum threshold value of 65%. Almost all fish products and recipes had good feedback from sensory evaluation because of their good taste, aroma, and gastronomic pleasure, as shown in Table.1. The sensory scores in P4, P5, and R7 groups were more than 80%, even more than 90% in R9 and R10 groups. Conversely, the Carp miso soup recipe was discarded because of the lower sensory evaluation value (65% <).

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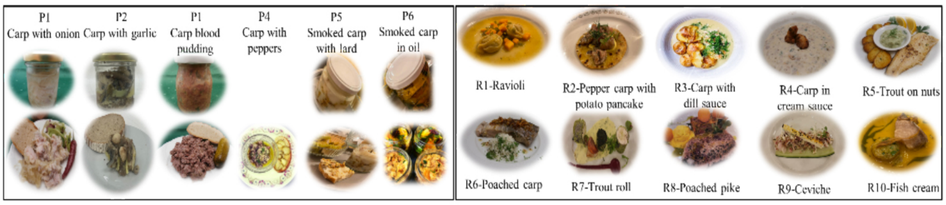
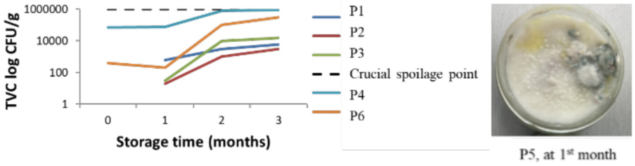


Fig.1. Fish products and dishes



P5, at 1st month

Fig.2. The change of TVC in fish products during storage (4°C).

Table 1. The nutritional composition and the content of fatty acids, sensory scores, and material prices of fish products and dishes.

	P1	P2	P3	P4	P5	P6	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
Proteins (%)	12.2	11.8	10.2	9.5	13.1	13.1	9.6	8.9	5.6	1.8	15.4	13.9	14.0	14.0	12.3	19.2
Fat (%)	19.2	15.8	14.5	39.0	61.8	49.3	7.6	19.7	5.9	0.5	5.8	21.8	6.6	13.9	7.3	8.3
NaCl (g/100g)	1.4	0.5	1.2	1.5	2.1	1.5	1.2	0.9	0.5	0.8	0.7	0.8	0.3	0.9	1.2	1.9
EPA+DHA mg / 100g	78.5	121.8	56.5	87.2	21.5	46.0	17.0	82.0	53.0	32.0	320.0	65.0	300.0	77.0	323.0	85.0
n-6/n-3	2.2	2.5	2.2	4.9	5.4	2.2	8.8	4.9	2.7	2.4	3.1	2.1	2.2	3.6	3.2	4.2
Sensory score (%)	76.4	74.8	77.6	73.6	80.5	83.2	65.5	77.7	73.6	75.9	69.0	69.3	88.6	65.0	90.9	93.6
Price -material (€)	1.30	1.31	1.05	1.36	1.72	1.74	0.56	2.10	2.69	1.44	2.36	5.36	4.49	5.10	3.34	3.69

From the economic point of view, the prices of these new products and recipes were acceptable for consumers. This study achieved utilization of the unpopular parts of fish, such as fishtails and filleting skeletons. Especially, smoked carp in oil, carp blood pudding, ravioli, and poached pike achieved utilization of the unused fish biomass and decreased the losses of the fish biomass. The authors encourage the fish factories and restaurants to develop new fish products with the other tissues and organs (like fish skin, fins, tails, heads, liver, and gonads) to increase the diversity of fish products and produce the new products with the low prices for the enhancement of fish consumption and improving the eating habits of humans. Apart from the above advantages, the utilization of fish biomass can produce economic fish products and even avoid the cost of handling unused fish parts.

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NUMERICAL MODELLING OF A TYPICAL MARINE OPERATION SCENARIO IN AQUACULTURE, WITH RIFLEX AND SIMO IN SIMA WORKBENCH

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Introduction

Aquaculture of Atlantic salmon in coastal and offshore locations is an operation intensive industry. This paper first gives a brief overview of the most typical marine operations in the aquaculture industry. With the aquaculture industry developing toward offshore locations and the need to ensure efficient and safe operations, being able to predict the operation performance in planning phase and to monitor and assist the actual operation in real-time becomes more and more important.

To meet these needs, the paper focuses on a typical lifting operation over the side of a standby working vessel with onboard crane and establishes a numerical model for the complete system using RIFLEX and SIMO in the SIMA workbench. The working vessel is moored to the mooring system of the cage. The hydrodynamic performance of the cage -vessel system and the connection line tension during the lifting operation is evaluated in irregular wave and current conditions. At the end, the performance of the cage-boat system during the lifting operation will be summarized for different sea states and weather directions.

The cage and standby working vessel system and numerical modelling

A scratch of the cage – vessel system including the cage's mooring system is illustrated in Figure 1.

In this paper, the operation scenario of one service boat moored to a fish cage is numerically simulated. The simulation is performed in SINTEF Ocean's Integrated Simulation Environment, SIMA.

Fish cage and its mooring system model is made in RIFLEX in SIMA. RIFLEX solves the nonlinear hydrodynamic/structural responses of slender marine structures in a sea environment using FEM method. Special net elements in RIFLEX are applied to model the fish nets. In addition to standard cross section input parameters such as mass, volume, stiffness, damping and hydrodynamic force coefficients, fish net elements also require special input parameters such as solidity ratio, current velocity reduction coefficient and net width specification. The floater and bottom ring of the cage are modelled using beam elements. The bridle lines, frame lines and mooring lines are modelled using bar elements.

Hydrodynamic coefficients for the service vessel are calculated by WAMIT, which are then imported to SIMO inside SIMA and further integrated into the RIFLEX model of the fish cage to perform coupled SIMO-RIFLEX simulations. SIMO is a program to perform frequency and time domain hydrodynamic response analysis of rigid bodies in a sea environment, it can also be used to perform the same type of analysis for multi-body system and marine operations.

The service vessel is moored to the cage by four mooring lines: two connected to the cage floater and two connected to the buoys. The connection lines are modelled using bar elements in RIFLEX.

Numerical simulation

Static analysis is performed in current condition. Dynamic analysis is performed in irregular wave and current condition. Hydrodynamic interaction between boat and cage is so far not considered and uniform current filed is assumed. Wind load is not considered in this work. The simulations to be performed are summarized in Table 1.

Numerical simulation of operation scenario provides useful information for the planning and execution of corresponding marine operations. It can for example estimate the time window for a particular operation. Through digital twin of onsite measurement, the numerical model can be tuned to give as accurate prediction as possible, the numerical model can also be used to give real time visualization of the system response by reading in onsite measurement data in future.

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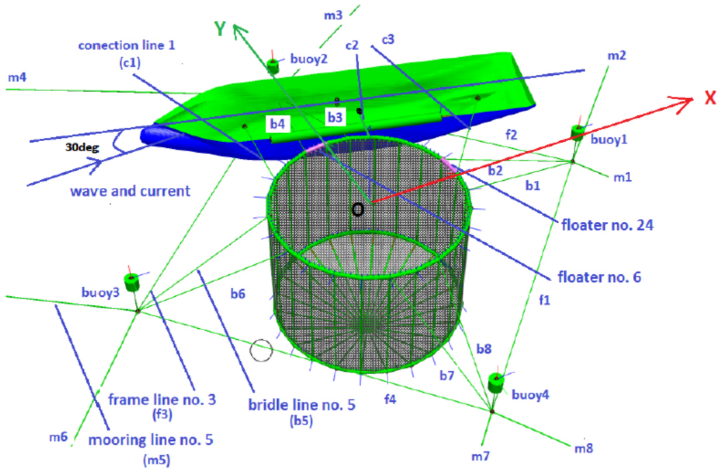

Figure 1: Illustration of cage – vessel system

Table 1: Illustration of cage – vessel system

Static analysis	
Current velocity (m/s)	0.1, 0.2 and 0.5
Current direction (deg)	210deg, 240deg,270deg, 300deg, 330deg
Dynamic analysis	
Significant wave height (m)	0.5
Peak period of wave (s)	5, 8, 10
Wave and current direction (deg)	210deg, 240deg,270deg, 300deg, 330deg
Current velocity (m/s)	0.1, 0.2

COCKLES (*Cerastoderma edule*) AQUACULTURE: COMPARISON OF CULTURE IN INTERTIDAL SHELLFISH PLOTS VS SUSPENDED CULTURE

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Introduction

Cerastoderma edule is widely exploited and supports important commercial bivalve fisheries throughout its geographical range, particularly in Atlantic Area of Europe. However, the synergistic action of fishing pressure coupled with the rapid growth rate, short lifespan and huge mortality by diseases of *C. edule* leads to large inter-annual fluctuations in stock abundance and periodic recruitment failure. The development of cockle aquaculture and restocking programs could be an efficient fishery management strategy in order to rebuild stocks. To achieve this purpose, cockles aquaculture was tested, by transplanting juveniles from natural cockle beds to growth in different culture systems.

Material and methods

Two different outdoor culture systems were tested: in intertidal shellfish plots in a bivalve production park (37°01’25’’N; 07°48’41’’W) and in a suspended structure in earthen ponds tanks used for fish production in polyculture (37°01’54’’N; 07°49’17’’W) in Ria Formosa lagoon, south of Portugal. Juveniles from natural cockle beds of Ria Formosa with an initial size of 19 ± 2mm and 2.2 ± 0.7g by weight were used in all trials. Survival, growth (biometric measures - length, width, thickness and weight) and condition index (Walne & Mann, 1975) were monthly evaluated. Two different expected final densities were tested in both systems: 2 and 3 kg.m^{-2}. For each density, the experimental area of intertidal shellfish plot was composed by 6 squares 1x1m and half of the squares were protected with a plastic net with a grid 5x5mm to avoid predation. In the earthen ponds, two different containers for suspended cockle culture hanging from a cable (long-line) with floaters, set side by side alternating the two different densities were tested: rigid mesh bags and circular perforated (1 cm mesh) trays with 40 cm in diameter and 10 cm in height. Theses trays were piled, allowing comparison of culture at two depths (20 and 40 cm).

Results and discussion

The on-growing experiments carried out in intertidal shellfish plots showed that net protection against predators increased survival between 15 and 20 % in the first month after restocking. The *C. edule* juvenile’s length growth rate was 60 μm per day in a mean period of 4 autumn/winter months, when the water temperature and food were lower. In general, the low density showed the best results. In this conditions and without net, biomass yield was around 1,400 g.m^{-2} and individuals reached maximum values of 25.71±2.94 mm of mean length and 5.31±1.38 g of mean fresh weight. At the end of the experiment, the cockles condition index showed similar values for all the tested rearing conditions.

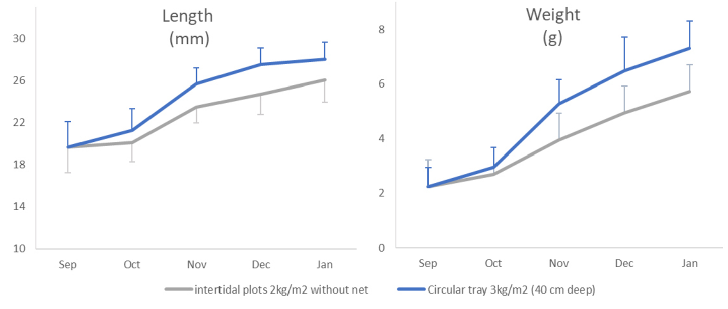

Figure 1 – The best growths of *Cerastoderma edule* juveniles (length and weight) in intertidal shellfish plots vs suspended culture.

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Growth of cockles reared in suspended culture was significantly better than the growth of cockles reared in intertidal shellfish plots. In the same period, length growth rate of cockle reared in the circular trays at 3 kg.m^{-2} can reach 85 μm per day (27.83±1.69 mm length and 6.92±1.30 g of weight at the end of the experiment). In rigid mesh bags, growth was slower (25.22±1.91 mm length and 5.29±1.05 g of weight in 3 kg.m^{-2} density at the end of the experiment). Biomass yield in circular trays and bags varied between 1300g.m^{-2} and 1900 g.m^{-2}, and the effect of density and depth of rearing were not substantially significant. Condition index of cockles reared in suspended culture was substantially higher and best results were found in November for cockles reared in circular trays placed at 40 cm of depth within the higher density.

The high cockle's growth found in suspended culture compared with intertidal shellfish plot culture may be explained by the highest input of organic nutrient that can exist in polyculture ponds due to the addition of fish food. On the other hand, in this ecosystem, cockles were continuously immersed with constant food availability which can be advantageous, compared with the intertidal plots where cockles are exposed to long periods of immersion. In spite of achieving greater growth increments in the culture in earth ponds tanks, cockles presented shell deformations resulting from the lack of sediment. However, these shell deformations do not increase mortality.

Results showed that was possible to attained commercial size in few months with high densities, in both rearing systems. In conclusion, juvenile's cockles reared in intertidal shellfish plots and in a suspended system in earthen ponds are both viable. The supremacy obtained of individual's growth in the suspended culture is undermined by deformations that can decrease the quality of the product and create obstacles to their sale directly to the consumer. So, this product can be forwarding to cannery industry or proceeded food without shell and cockles reared in intertidal plot is more targeted to fresh consume.

Acknowledgements
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BIO-FARMING FOR BIOACTIVE COMPOUNDS: SCREENING OF NORDIC HERBS AND UNDERSTANDING THEIR POTENTIAL SYNERGISM WITH BIOACTIVE COMPOUNDS AGAINST ATLANTIC SALMON PATHOGENS

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Background

Aquaculture production in Norway has grown immensely in terms of quantity and value compared to other developed nations in the last decade [1]. This tremendous growth depends directly on fish feed and fish health along with other best management practices contributing to fish welfare. Also, chemical compounds used in aquaculture have increased concomitantly and have been used widely for feed and health management. However, a further increase in aquaculture should occur in a sustainable manner without harming the environment or posing a threat to biodiversity and consumer safety. This can be achieved by replacing synthetic compounds, such as the antioxidant (eg. ethoxyquin) and antibiotics with natural, efficient, non-toxic alternatives identified among bioactive compounds from plant-based sources.

In the present study, we have screened 339 extracts from various plant parts of different varieties of 9 herbs (*Artemisia annua*, *Achillea millefolium*, *Rhodiola rosea*, *Mentha piperita*, *Oreganum vulgare*, *Humulus lupulus*, *Leuzea carthamoides*, *Rosmarinus officinalis*, *Portulaca oleracea*) grown in Norway for antioxidant (AO) property and antimicrobial (AM) activity against the Atlantic salmon pathogens, *Aliivibrio* spp. *Moritella* spp. and *Tenacibaculum* spp. The selected pathogens mainly cause ulcerative disease in salmonids, which is among the main reasons for reduced welfare and increased mortality in on-growing salmon in Norway [2]. Furthermore, intensive farming conditions can lead to increased bacterial and parasitic diseases, which in turn lead to huge losses in farmed fish [3]. The objective of this study was first to assess the bioactivity of herbs produced in Norway based on evaluation and comparison of the antioxidant and antimicrobial properties of the plant extracts, and second to select the potential phytogenic compound or whole extract that revealed the best properties to be included as an additive in fish feed.

Methods

The herbs were grown in open field or in green house in Norway. The extracts were prepared by successive extraction with three different extraction solvents: dichloromethane (DCM), ethanol and water. The commonly used antioxidant methods, ABTS and Folin Ciocalteu, were used to measure the antioxidant capacity of the extracts. Agar disc diffusion assay was used to test the susceptibility of bacterial strains to extracts. Tested strains were grown in two different growth media mimicking external sea water environment and internal tissue of salmon. Extracts that revealed the highest AO and AM activities were tested in salmon skin cells *in vitro* (keratocytes) and cytotoxic activity assessed.

Results

Among 9 herbs studied, *Origanum vulgare*, *Artemisia annua*, *Humulus lupulus* and *Rosmarinus officinalis* had an interesting effect on AO and AM properties. The DCM extracts of the selected plants revealed particular inhibition of the salmon pathogens. However, the extracts were found to have a lethal effect on salmon keratocytes in a dose-dependent manner. Therefore, further studies are required to fully understand its suitability as a natural additive in fish feed and its impact on fish. Our study also indicated that the bioactivity may be caused by a combination of compounds present in the whole extracts, which cannot be obtained using purified individual compounds from the same plant.

Conclusion

The results from screening of Nordic herb extracts using salmon pathogens and salmon keratocytes show a great potential in selecting a suitable herbal extract/compound that can be used in the salmon farming. Moreover, plant extracts or individual bioactive compounds may have additional benefits other than AO and AM activities, but might also show adverse effects.

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BIOSENSOR TECHNOLOGY FOR OYSTERS BEHAVIOUR ASSESSMENT IN BIOFLOC ENVIRONMENTS

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Introduction

Bivalve mollusks are sentinel animals sensitive to the quality of aquatic environments (Pereira et al., 2014). Variations in physico-chemical parameters or presence of contaminants may lead these animals to change their behavioural patterns (Newton et al., 2007). Since oysters and mussels open and close their valves to perform physiological activities, the stress promoted by environmental factors may affect shell movement patterns (Hartmann et al., 2016).

By understanding the behaviour of bivalve mollusks, it becomes possible to employ them as biosensors for water quality conditions. In integrated multi-trophic aquaculture (IMTA), these organisms have commercial value and may take part in the chain as filtering animals (Crab et al., 2012). This work proposes to assess the behaviour of *Crassostrea gasar* oysters in IMTA environments containing biofloc in order to evaluate its application as a biosensor in aquaculture sites.

Materials and Methods

An experiment employing *C. gasar* oysters in biofloc water from shrimp breeding tanks was performed. The setup comprised instrumented animals disposed inside water tanks and an electronic acquisition system to log behavioural readings.

Oysters were instrumented with neodymium magnets and Hall Effect sensors, each fixed to opposing valves. The attachment of the respective devices was made using epoxy resin and cyanoacrylate glue for achieving greater resistance during tests. A 3-core cable was used to connect each sensor to the acquisition system and provide power (5V).

Water tanks (100 L) were employed as recipients for the animals. A nylon net was fixed at half height within the tanks to hold oysters in order to avoid their contact with sediments. Three biofloc concentrations and a control were tested (control, low - up to 100 mg/L, medium - between 100 to 200 mg/L, high - above 200 mg/L). Proposed concentrations were based on the range for optimized shrimp growth (Gaona et al., 2017). Each test tank contained four instrumented oysters. Temperature was controlled during the experiment and all tanks were connected to a continuous recirculation system to avoid precipitation of particulate matter. The experiment was performed in triplicate.

The acquisition system was composed of two multiplexing boards connected to an Arduino MEGA microcontroller. This system logged data at 2 Hz rate to an SD card as CSV files. After pre-processing, data was obtained as the percentage of valve opening for each animal. The experiment was performed in triplicate throughout a 28-day period. Figure 1 depicts the experimental setup.

Results and Discussions

Behavioural data from 28 days of recording were split into 4 weeks. Every 120 readings were grouped into a 1-minute value. Data analysis proceeded observing minute data frequency for every week. Statistical analysis was performed using ANOVA with 5% confidence level. The control group presented higher overall average opening amplitude than all three biofloc concentrations. This observation shows the particles may stress the bivalves, promoting longer closed shell periods.

In the second and third experimental week, the opening frequency showed significant differences among groups. Higher frequency of open periods was observed for the control group when compared to biofloc groups, especially at high concentration. Higher concentrations recorded longer closed valve periods. By the third week, low and medium groups presented similar opening frequencies when compared to the control.

Conclusions

The assessment of oyster behaviour in biofloc environments was observed over several weeks. Results show behavioural pattern variations with increasing particle concentration. Significant stress was observed at the highest concentration tested when compared to other groups. This behaviour may provide a feasible biosensor based on bivalve mollusks for IMTA environments.

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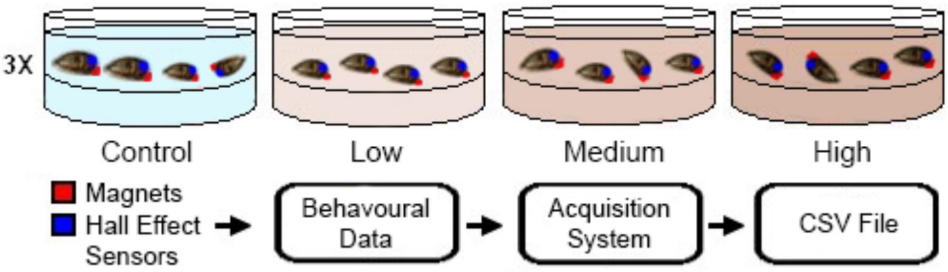

Figure 1 – Experimental setup for behavioural assessment of oysters.

Acknowledgements

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GENASSEMBLAGE 2.0 SOFTWARE AS A TOOL FOR MAINTAINING GENETIC VARIATION AND IN OPTIMIZATION AND MANAGEMENT THE BANKS OF CRYOPRESERVED STURGEON SEMEN

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Introduction

Highly polymorphic fragments of DNA, such as microsatellites, can be used to prepare genetic profiles of fish that could be used for breeding and gamete banking. These profiles can be used to identify individuals that are as different as possible and in management of the genetic variation in gamete banks.

Maintaining high genetic variation, expressed as high heterozygosity and allelic diversity, is crucial for populations viability of sturgeon fish and their adaptation to environmental changes. Conserving genetic diversity in broodstocks is important for conservation of all human-dependent fish species.

Identification genetic differences between sturgeons and optimization assemblage them in breeding pairs is very important for conservation biology and aquaculture of those species. If individuals are paired without knowledge of the genetic differences between them, they might be genetically similar, which would prevent optimal transfer of genetic variation from parental stock to their progeny. To help identify the best possible breeding pairs within and between breeding stocks and manage genetic variation deposited in gamete bank, Genassemblage 2.0 was constructed. The software is intended to be useful in the conservation of human dependent fish species (including sturgeons and paddlefish), the maintenance of genetic variation in broodstock, the production of juveniles for fish farming, and for educational purposes

Cryopreservation of gametes is used to protect the genetic resources of a fish population and to safeguard the level of genetic variability in the event of a catastrophe resulting in the extinction of the population. It is used for both endangered sturgeon species and economically valuable breeds and breeding lines.

The potential usefulness of cryopreserved gametes in protecting the genetic variability of sturgeons depends on the genetic diversity of the individuals from which the gametes were collected. The genetic diversity of a gamete bank can be managed if the genetic profiles of the gamete donors are known and a tool is available to process this information. To address this problem, we developed a tool that enables management of genetic variation in a gamete bank and added it as a new module for Genassemblage 2.0 software.

Material and methods

Genassemblage 2.0 is a free-of-charge Windows-based program. This software has four modules, which can be chosen by clicking on their respective bars. Its installer and a detailed user guide can be downloaded from the author's website. The software requires the use of MS Excel, version 2003 or newer and Microsoft .NET 4 framework.

An input file for Genassemblage 2.0 can be prepared in a MS Excel spreadsheet. Modules one and two described below use the same input file, but module three uses an slightly different input file than previous two. Details of constructing input files are given in user guide.

The module "Select best breeding pair" was designed for breeding individuals. The user can decide how many pairs to include in the set of best pairs. The suitability of pairs for breeding is evaluated by simultaneously comparing heterozygosity and percentage of weak heterozygotes expected in the progeny, as well as the number of different alleles that will be inherited by their offspring. In version 2.0, we have added the "*v* index", so that users can define the relative importance of the three indicators. To generate the results, we used the "Select best breeding pair" module and checked the fields for heterozygosity, percentage of weak heterozygotes and number of different alleles expected in the progeny. Using the *v* index, we defined the relative importance of the indicators as heterozygosity (0.40), share of "weak heterozygotes" (0.20) and number of different alleles (0.40). The number of pairs was set at four.

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The module “Select individuals for group spawning” is new in Genassemblage 2.0 and was intended especially as a help in breeding of fish. It enable to find a group males and females that, when bred together, will contribute the highest genetic diversity to the progeny. After clicking on the bar of this module, the user can indicate how many males and females to include in this set. In the example presented bellow we used this module to identify a set of two males and two females that will contribute the highest allelic diversity to their progeny. In the window of this module we chose “Number of alleles” as the primary indicator.

The module “Management of genetic variation in gamete bank” is based on allelic diversity across all loci included in genetic profiles. It calculates the overall number of alleles across all loci and indicates which samples should be used to obtain a target percentage of allelic diversity while using as few samples as possible. After the user loads the input file, the program will automatically calculate the number of alleles across all samples. In the line “minimum level of genetic variation”, the user should enter the percentage of total genetic variation deposited across all samples and loci that should be in the group of samples chosen by the software. In the example presented below, this value is 100%; as a result, all alleles included in the input file are included in the group of selected samples.

The details of calculations performed by those three modules are given in user guide.

Results

The results provided by all modules are presented in MS Excel files. In module “Select best breeding pair” they include four sheets: the expected heterozygosity in the progeny of each pair, the number of alleles that will be inherited by progeny of weak heterozygotes and the results of the *v* index. Based on this index, we found four pairs that were selected as optimal for breeding because their progeny have a high heterozygosity, low share of weak heterozygotes and high number of alleles.

The results of “Select individuals for group spawning” module indicated optimal group of males and females. Their progeny will inherit an highest number of alleles among all variants of male and female groups. Moreover their progeny will be highly heterozygotic.

The result file of module “Management of genetic variation include lists the alleles at each locus, and the alleles across all samples and loci. Transferring 100% of the allelic diversity detected in this group of samples can be done by using just a few samples across all included in the input file. Their names and genetic profiles are presented in the output file.

Conclusion

Genassemblage 2.0 identifies the set of parents that have the best values of genetic variation indicators. It indicates optimal sets of breeding pairs without using the same individuals more than once, thus reducing inbreeding risk. It can also select individuals for group breeding of fish to transfer the highest amount of genetic variation to the next generation. As calculations show, it is possible to use a fraction of the samples stored in a gamete bank and transfer the same number of detected alleles as when using all the samples. In this way, Genassemblage 2.0 can be in management of the genetic variation resources deposited in a gamete bank and can help in assemblage fish for breeding in fish farming and conservation of human dependent populations of sturgeons.

REUSABLE, RECYCLABLE AND SUSTAINABLE SEAFOOD PACKAGING FOR AQUACULTURE PRODUCERS

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Introduction

Ocean pollution is affecting our marine environment in unprecedented ways. Eight million metric tons of plastics are dumped into our oceans annually, many of which take 500-1000 years to degrade. Polystyrene (EPS) boxes used to pack fresh fish are a key contributor to this due to significant volume and market share, combined with single-use behaviour and low levels of recycling capability amongst its users. Governments around the world enacting climate-change policy are beginning the process of outlawing EPS which has accelerated the adoption of new alternatives. The change to more sustainable packaging has been a case of the ‘chicken or the egg’ in terms of whether it has been led on the producer or customer end. In many cases, producers looking to follow through on ESG standards have adopted packaging that meets their internal environmental goals, while the customers and receivers of such stock including secondary processors, wholesalers and retailers have fast-tracked the process by demanding they receive products in recyclable, reusable and overarchingly sustainable packaging.

Our solution

Disruptive Packaging is an Australian inventor, manufacturer and distributor of sustainable packaging with the goal of addressing the aforementioned concerns. UNICOR is a revolutionary alternative to EPS and waxed cardboard with key environmental and performance benefits. The product has been engineered to be fit-for-purpose for the seafood industry taking into account multiple factors. First, UNICOR is 100% waterproof, leak proof and washable. Unlike cardboard alternatives, UNICOR stands up to the test of the wet nature of fish and can withstand any moisture exposure throughout the supply chain. Further to this, UNICOR strengthens in the cold chain as a result of its chemical composition by gaining greater rigidity. Additionally, UNICOOL is a strong insulator and has thermal properties that ensure that the integrity and quality of the seafood packed inside is maintained for good periods of time. Understanding that different species require different packaging solutions, UNICOR is fully customisable in shape, thickness, size and format (lid & base vs. single piece). Furthermore, a magazine quality print creates maximum brand exposure and gives customers a unique opportunity to use their packaging as a means of marketing. Finally, due to its flat packed nature, UNICOR eliminates the need for mass storage space and therefore reduces both warehouse and shipping costs.

UNICOR has been designed with sustainability and the environment at the core of its purpose. Unlike EPS, UNICOR is a vehicle that will help achieve key European objectives such as the Farm to Fork Strategy and European Green Deal. The product has been designed to directly address and eliminate the harmful effect EPS and waxed cardboard have on our oceans and planet. By being 100% recyclable, UNICOR supports the circular economy by ensuring no packaging ends up in landfill or the oceans as is the case with EPS. Its reusability due to waterproof properties allowing for washing means overall consumption of boxes by volume is reduced. Further to this, UNICOR is made up of at least 65% natural raw Earth elements that are sustainable in supply and, UNICOR does not contribute to deforestation. The carbon footprint of UNICOR is also significantly lower than its EPS and Cardboard alternatives by reducing overall CO2 emissions by 80%. Furthemore, the energy required for production is lowered by 25% and recycling by 92%.

Conclusion

If the Blue Economy is to truly be realised, action must be taken today to ensure our oceans are able to provide high quality seafood. Marine pollution puts this at risk and until harmful packaging such as EPS and waxed cardboard are eliminated, initiatives such as the European Green Deal and Farm to Fork Strategy will be at risk. Encouragingly however, the shift has already begun and aquaculture producers now have an alternative packaging option in the form of UNICOR that provides superior performance specially designed for seafood, as well as significant environmental benefits.

MICRO-RNA RECRUITMENT IN GREATER AMBERJACK'S (*Seriola dumerili*) DEVELOPMENTAL STAGES UNDER DIFFERENT REARING TEMPERATURES AND DIET CHALLENGES

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Introduction

The last decades, the greater amberjack (*Seriola dumerili*), has been considered a strong candidate as a newly introduced species in the farming industry. Principally, its high-quality meat, as well as its significant growth rate, high commercial value, and its cosmopolitan distribution, meets a lot of the aquaculture sector requirements. However, dysfunctions regarding greater amberjack's reproduction, egg survival and growth, limits the establishment of the species in the farming industry.

Among others, temperature shifts, as well diet variations have an impact on diverse physiological processes in teleosts, many of which are regulated by micro-RNA (miRNA). miRNAs, as a member of the epigenetics mechanisms, may alter gene expression -in this case post-transcriptionally- without altering the gene sequence. So, as to reveal the underpinning molecular processes of the critical period of embryo and larval development of greater amberjack, their miRNA repertoire under different rearing temperature, and different diet regime was searched in the current study.

Material & methods

Greater amberjack eggs were transferred to the HCMR Aqualabs facilities where they were kept at 20°C or 24°C. At the first feeding stage, larvae were further separated into two groups, one fed with rotifers and the other fed with copepods (Figure 1). Samples at a stage before first feeding and at the flexion and mid-metamorphosis stage were collected. Total RNA was extracted, and miRNA libraries were constructed and sequenced at an Illumina platform.

Results/ Discussion

The number of the sequencing reads for each condition ranged from ~10 to ~29 million reads. After trimming, the sequence length analysis revealed that most of the reads were 22 bp long (Figure 2), which is the mean length of miRNAs.

Principal component analysis located the replicates of the same condition close to each other. Searching the most abundant miRNAs, as well as the most differentially expressed miRNAs, showed higher variation between different stages reared under same conditions (temperature and diet). The later findings may indicate the strategic role of the miRNA in the fulfillment of the developmental processes, as well as the necessity of a specific miRNA repertoire for each developmental stage.

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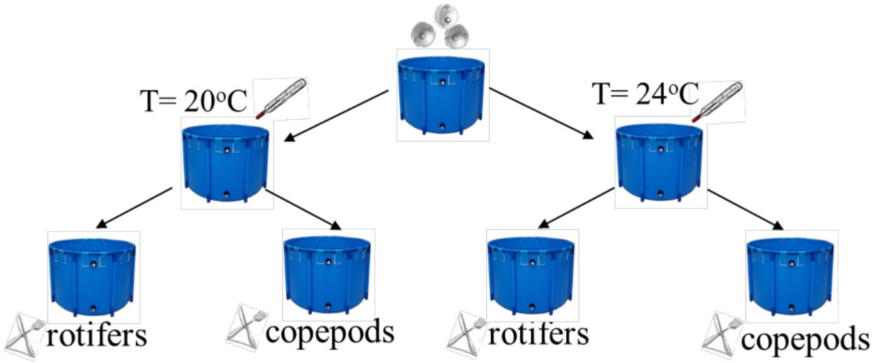

Figure 1: The experimental design of the present study.

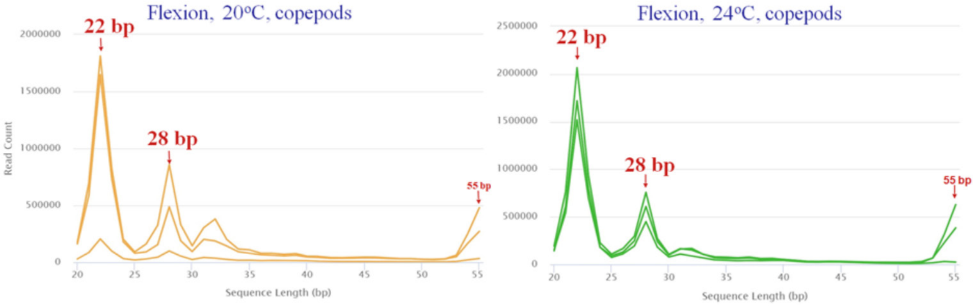

Figure 2: Size distribution of the sequencing reads, after quality and adaptor trimming.

EVALUATION OF ECOLOGICAL STATUS AND FISHERY MANAGEMENT OF EUROPEAN PONDS: A META-ANALYSIS

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Introduction

Central European fishponds are shallow artificial water bodies of different sizes (<1 ha to several hundred ha) used for aquaculture (mainly fish production of common carp) since the Middle Ages. During the last century, farming in the fishponds has undergone intensification and diversification of fish production. Long-term excessive nutrient loads have modified biological processes such as primary production, nutrient utilization, respiration, or biogeochemical cycles. Overall biodiversity loss, excessive cyanobacterial blooming, and often hypoxia have been increasingly recorded in many fishponds. Such changes indicate progressing deterioration of fishponds with ultimate impacts on both fish production and freshwater environments.

Phosphorus (P) has been reported as the superior limiting nutrient in freshwaters that determines the status of water bodies and supports primary and secondary production. Yet in shallow P-rich lakes and ponds, denitrification has been recognized as significant nitrogen (N) loss driving phytoplankton from P to N limitation. The stoichiometric imbalance might be reinforced by internal P loading from anoxic sediments and driven by fish bioturbation. Ptacnik et al. (2010) recommended the dissolved inorganic N to total P ratio (DIN:TP) as the best indicator for inferring in situ N vs. P limitation from chemical data in nutrient-rich freshwaters. This study examines the current nutrient status of Czech fishponds to reveal their actual nutrient limitation. We hypothesized that the nutrient regime might vary on both temporal and spatial scales.

Material and methods

Our results covered 33 Czech fishponds from lowlands (<200 m a.s.l.; n = 6 ponds), moderate altitude (200–450 m a.s.l.; n = 19), and highland region (>450 m a.s.l.; n = 7). The ponds were monitored irregularly on a yearly basis from 2007 to 2021 (resulting in a total of 180 pond-year cases).

The search criteria were designed to focus on month-wise data (from April to September) with known fish production. The main goal was to analyze physical and chemical parameters and management data (fish production and supplementary feed supply). The data were further split into three phases of the growing season: spring (April–May; water temperature <14°C), midsummer (June–July; maximum primary production, rapid fish growth), and late summer (August–September; apparent over-exploitation of natural food, frequent oxygen deficits, stagnant fish growth). The data were analyzed using linear regression models and a multivariate approach. Multivariate analyses were used to determine relationships among environmental and fishpond landscape characteristics, including management interventions (mean depth, altitude, feeding, final fish production).

Results

Studied fishponds were classified as mesotrophic to hypertrophic with high nutrient concentrations (median (range) TN: 2 (0.3–9) mg/L; TP: 150 (8–1300) μg/L), resulting in high phytoplankton biomass (median (range) chlorophyll *a* concentration: 70 (1–760) μg/L), and low transparency (median (range) Secchi depth: 50 (5–350) cm).

Multivariate analysis revealed that feeding was positively correlated with fish production while negatively with the DIN:TP ratio. Stoichiometry of available macronutrients showed high variability both on spatial and temporal scales. Despite high total N concentrations, we found decreasing trends in DIN (NH_4-N + NO_3-N) concentrations during the growing seasons. The relationship between chlorophyll *a* and DIN:TP suggests that all fishponds at lower altitudes (<450 m a.s.l.) likely tended to N limitation from mid- to late summer, while ponds at higher altitudes (>450 m a.s.l.) apparently remained P-limited regardless of the season phase.

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Discussion

Results of data compiled from studies carried out over various temporal and spatial scales suggest that fishpond might be stressed by seasonal and spatial stochiometric imbalances of available macronutrients.

Wholepond fertilizing „experiments" held in the last century in Czechia suggested P as a nutrient limiting fish production (Pechar et al. 2002). The positive results of P addition were reflected in fish production increment and naturally drew attention away from N availability because, in mesotrophic ponds at that time, P certainly was the limiting resource, unlike in eutrophic ponds at present. Supplementary feeding (e.g., cereals rich in phytate P) seems to be connected to the DIN:TP shift in mid- and late summer. Contrary to cyanobacteria, many algal species are sensitive to a severe shift in stoichiometry balance and unable to grow under N limitation.

Ponds are conceived as a black box with inputs and outputs of nutrients, but little attention has been paid to the process involved and stoichiometry of these inputs and its further manipulation to achieve the desired goal (i.e., good water quality and fish production). The current pond management practices deserve a revision, as warranted by changing nutrient status, stoichiometry, and climate. One of the concepts is the balanced pond feeding concept or nutritious pond concept (e.g., Kabir et al. 2020, Roy et al. 2022) based on a "smart nutrition" and fish bioenergetics aiming to keep current fish production while maintaining good water quality. Such a concept, presently under field evaluation, could manipulate not only the nutrients supplied to fish but also those released *in situ* by fish. For instance, supplementing the missing N (essential amino acids) and reducing the P (phytate) released from fish in late summer is one such strategy (Roy et al. 2022).

Acknowledgements

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AN AQUACULTURE 5G DECISION-SUPPORT SYSTEM FOR MONITORING AND IMPROVING FISH PRODUCTION

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Introduction

Marine aquaculture in Europe is evolving into a high-tech industry, with many modern aquaculture systems incorporating the collection of heterogeneous data from multiple sources into their daily routines. Globally, aquaculture and fisheries production are an important source of accessible nutritious food, and form a basis for livelihoods. Fish consumption account for 15-20% of the protein consumption of 2.9 billion people worldwide. They also play a key driving role of coastal and rural economic well-being. However, environmental impacts, fish welfare, and the combined effects of ocean-based activities and climate change on resources and ecosystems, put the sector at risk and undermine the resilience of those relying on it. Improving fisheries and aquaculture management is thus crucial to ensure sustainability and that future generations continue to benefit from ocean resources and ecosystem services.

5G-HEART (5G for Health, Aquaculture and Transport) is an EU project which aims to offer new solutions through advances in 5G technology to facilitate sustainable development. Within the aquaculture sector this includes better environmental monitoring, waste management and improved fish monitoring and performance. Technologies such as the legacy 2G network and current 3G/4G technologies are particularly suited for most current requirements but can be constrained in terms of coverage and data rate. More advanced use cases will require high reliability, low latency, global coverage, energy efficiency and the ability to connect a large number of sensors/devices without loss of performance. All these requirements should be met through the 5G infrastructure.

Methodology

In Greece, the solution proposed aims to cover daily operations, such as automated monitoring of fish health and welfare, optimal feeding and infrastructure integrity, in collaboration with a commercial sea bass and sea bream producer, Skironis SA located in Megara, Athens.

The project aims to develop a complete decision-support solution which provides improved monitoring and management of fish farming operations and planning of production. Part of the solution is the platform (web interface) consisting of two main elements:

1. Environmental (water quality) and stock (fish numbers, feeding rate, health) monitoring in order to optimize daily activities,
2. Supporting management decision making, with proposals based on the possibilities offered by the internet of things (IoT) and artificial intelligence (AI).

Expected Outputs

The project is evaluating key production indices focused on stock monitoring (number of fish per pen, fish behaviour and appearance), with the goal to reduce the number of mortalities, monitor feeding levels in order to optimise the feed conversion ratio (FCR) which will in turn reduce waste (less uneaten food pellets and reduced feed costs). Results on the number of live fish and on the FCR will be presented and assessed, in terms of how well the complete system offered from WINGS performs. Additional data collected on the performance of the 5G network will also be analysed to determine the potential benefits of this infrastructure.

Acknowledgements

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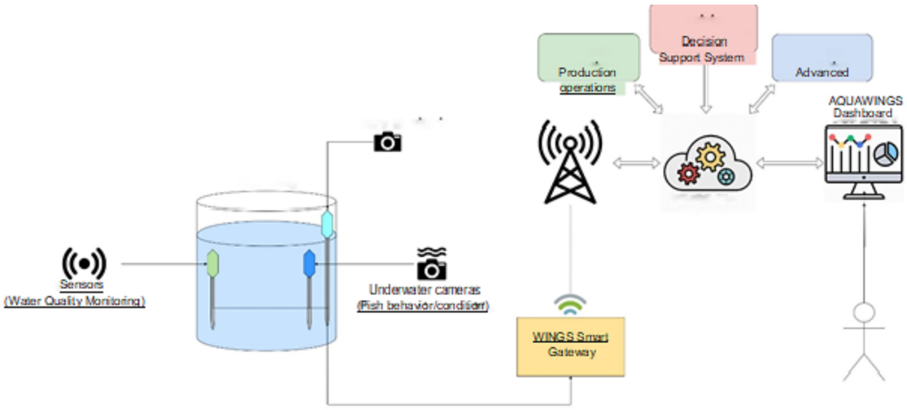

Figure 1: An illustration of the WINGS decision-support system used at SKIRONIS for the monitoring of the farm environment and stocked species performance.

CO-FEEDING DRY AND LIVE FEED IN FIRST-FEEDING EUROPEAN SEABASS *Dicentrarchus labrax*: EFFECTS ON FUNCTIONAL DEVELOPMENT OF THE DIGESTIVE SYSTEM, LARVAE AND POST-LARVAE PERFORMANCE

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Introduction

Marine larval feed technology is a fast changing field with great progress in manufacture technology. Many of the fish larval feed industries provide small enough feed particles that cover the nutritional needs of newly hatched larvae and have high acceptability and palatability, water stability and low nutrient leaching (Hardy and Barrows, 2002). In the hatchery practice of European seabass *Dicentrarchus labrax*, dry feed is progressively introduced to fish larvae not earlier than 25 days post hatching (dph) with the concomitant use of live feeds (rotifers, *Artemia*) until weaning. However, compared to live feed, dry feeds appropriately manufactured (i.e. physical and nutritional properties) are expected to better provide for all nutritional needs of altricial fish species, such as European seabass. Although the exclusive use of dry feeds is still inefficient, it is widely accepted that the longer the co-feeding period of live and dry feeds, the better the larvae performance at weaning (e.g. Cañavate and Fernández-Díaz, 1999; Khoa et al., 2020). The aim of the present study was to investigate the introduction of dry feed as early as on mouth opening (7 dph) on growth and functional development of the digestive system of European seabass larvae, as well as to monitor post-larvae growth performance and deformities.

Materials and methods

The experimental trial was conducted in two stages: Stage 1, Hatchery rearing (Hr), was performed in a commercial marine fish hatchery. Six tanks of 9 m^3 were stocked, two at a time, with eggs of the same broodstock. In three of the tanks a commercial dry feed (Larviva Prostart, Biomar) was introduced on 7 dph (DF7), while in the other three tanks the dry feed was introduced on 25 dph (DF25) according to a common hatchery protocol. In all experimental tanks, the larvae were fed with rotifers from 7 dph up to 15 dph and with *Artemia* nauplii/metanauplii from 15 dph up to 35 dph. From 36 dph larvae were fed dry feed only. Larvae samples were observed under microscope to confirm food consumption. During larval rearing, water quality was monitored daily and larvae were sampled daily from 7 to 17 dph, and at 5-days intervals up to 42 dph to estimate larvae length and digestive function (i.e. digestive enzymes specific activity). On 68-70 dph larvae of each Hr tank were graded to two size classes (Big, Small). Data obtained were used to calculate survival and performance. Stage 2, Laboratory rearing (Lr), was performed in a recirculating seawater system. On 61-63 dph ungraded larvae of each Hr tank were transferred to laboratory installations. On 64-66 dph, seven hundred and twenty (720) fish from each Hr tank were group weighed and randomly distributed in tetraplicated tanks (180 fish per tank). Fish growth (i.e. body mass, survival, specific growth rate-SGR, thermal growth coefficient-TGC, mass variation) was monitored for eight (8) weeks. All fish were fed the same commercial diet *ad libitum*. Water quality was monitored daily and fish were group weighed (app. 5-10 fish per group) every 15 days, while at the end of rearing fish were individually weighed. Phenotypic deformities were also individually recorded. During the last 15 days of rearing, food consumption was recorded to estimate feed efficiency (food conversion ratio-FCR).

Results and discussion

This is the third part of a general project concerning the early introduction of dry feed in first-feeding marine species larvae (Karakatsouli et al., 2019, 2021a, 2021b). Currently, European seabass Hr rearing has just been completed and Lr rearing is running. In parallel, laboratory work is going on to complete analysis. The present "incomplete" abstract will be replaced in due course.

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GERM CELL MANIPULATION AS A TOOL FOR ISOGENIC LINES PRODUCTION AND MANAGEMENT

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Introduction

Surrogate reproduction technology in fish has potential for aquaculture as well as preservation and propagation of endangered species or specific experimental lines. Some unfavourable biological characteristics for culturing such as a late maturation or a large body size are ideal candidates for surrogate reproduction using smaller and faster-maturing host. Therefore, surrogacy is well suited for isogenic line production in fish. Conventional methodology for isogenic line production is based on repeated uniparental inheritance to obtain doubled haploids. Each doubled haploid is in turn reproduced again by uniparental inheritance and isogenic line is produced. Although doubled haploid induction procedures have been developed in many fish species, only a few of them were successfully propagated to obtain isogenic lines and further maintained to facilitate their use in experiments. Main problems in isogenic line productions are yield of doubled haploid and their reproductive issues. Also, further interferences are needed to produce the next isogenic generations. We attempted to overcome disadvantages connected with the conventional approach for isogenic generation using germ cell manipulation involving cryopreservation and surrogate reproduction technology.

Materials and methods

Series of experiments were performed to develop optimal procedure for cryopreservation of common carp male and female early-stage germ cell. Mixture of permeating and nonpermeating cryoprotectants were used to firstly identify the optimal composition of the cryomedia. In turn, various freezing rates were tested to define optimal condition favouring germ cell survival.

The second step was verification of the suitable recipient for germ cells of common carp. We have employed ablation of primordial germ cells using injection of antisense oligonucleotide against dead end gene in goldfish. The goldfish were germ cell free. Therefore, we isolated single cell suspension from cryopreserved and fresh gonadal fragments using trypsin/collagenase dissociation, enriched with gradient sorting. Isolated cells were injected into the body cavities of sterilized goldfish and their gonadal development was monitored.

Goldfish surrogates were stimulated for spawning by controlled temperature and light regime. Collected gametes were used for fertilization and genotyping using carp and goldfish specific primers.

Results

Cryopreservation protocols for testicular and ovarian tissue based on DMSO cryoprotectant and slow rate freezing -1 °C/min yielded 40-60% post-thaw viability. Recovery and physiological activity of cryopreserved germ cells was confirmed after transplantation into sterile goldfish when cryopreserved germ cells retained colonization rate comparable to non-cryopreserved control confirmed by detection PKH26 labelling and RT-PCR. Afterwards, goldfish surrogates produced viable common carp progeny confirmed by genotyping and typical phenotype. Both male and female gametes were obtained, even from a single donor confirming feasibility of isogenic line production using transplantation from doubled haploid donor without necessity to reproduce given individual again by uniparental inheritance.

Discussion

Germ cell manipulation is potent biotechnology to ameliorate breeding of aquaculture species and preserve valuable genetic resources in environmentally relevant or even endangered species. This study aimed to introduce a novel and complex strategy capable to overcome shortcomings of the traditional procedure for isogenic lines production. The presented study developed and optimized protocols for common carp germ cell cryopreservation, therefore, the tissue from precious doubled haploid or even isogenic specimen can be safely stored in liquid nitrogen. Of the utmost importance, germ cell-free gonads of goldfish were confirmed to be suitable environment for common carp germ cells. Transplanted cells were able to colonize environment of goldfish genital ridge and differentiate into functional gametes giving rise to viable common carp progeny.

Funding

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EFFECT OF PHOSPHATIDYLCHOLINE FROM KRILL TOWARDS REDUCING LIPID MALABSORPTION SYNDROME IN ATLANTIC SALMON

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Introduction

Lipid malabsorption syndrome (LMS) is a pathological condition associated with limited lipid transport capacity in the enterocytes and results in excessive lipid accumulation in the intestine, most marked in the pyloric region. Recently published studies have demonstrated the importance of choline for prevention of LMS in farmed Atlantic salmon, and that choline levels in today's commercial salmon feeds are often below requirement levels. These studies indicate that a dietary choline level of about 3.4 g/kg is required to prevent LMS. The estimated requirement is based on studies with choline chloride as choline source. No similar documentation exits in which phosphatidylcholine (PC) is used as choline source. Krill meal (KM) is a sustainable source of PC, and the present study aimed to determine the effects of including KM or krill oil (KO), in which PC is the source of choline, for prevention of LMS and for growth in Atlantic salmon.

Material & methods

Two doses KM and KO (one low choline level- 1.5 g/kg; and one two suboptimal inclusion levels of choline-2.6 g/kg) were tested on Atlantic salmon of initial weight of 62g. These diets were tested against two reference diets, one un-supplemented, highly deficient with a choline level of 0.6 g/kg, and one supplemented with choline chloride to a level above the indicated optimum requirement, i.e., 4.0 g/kg. The fish were fed on their respective test diets for 8 weeks in freshwater, with replicate tanks/diet.

Results

After 8 weeks of feeding, the growth of the fish fed the KM and KO diets, at both levels, was significantly increased compared to the growth in fish fed the un-supplemented diet. These results indicate that PC from KM and KO included in the diet at a level resulting in diets with choline levels of about 1.5 g/kg satisfied the needs for growth. A clear dose-dependent, decreasing effect on the pyloric intestine somatic index (PISI) was observed for both KM and KO diets. No significant difference was observed regarding PISI for the fish fed the KM and KO diets with 2.6 g/kg choline and PISI of fish in the high choline reference diet. Accordingly, PC from both KM and KO significantly reduced lipid accumulation in the PI when added to a choline deficient diet. However, histological appearance as well as results from analyses of lipid content in the pyloric caeca, indicated that choline requirement is higher than 2.6 g/kg for diets in which KM and KO are the supplementary sources.

EFFECT OF KRILL MEAL ON THE REPRODUCTIVE PERFORMANCE OF BROODSTOCK NILE TILAPIA (*Oreochromis niloticus*)

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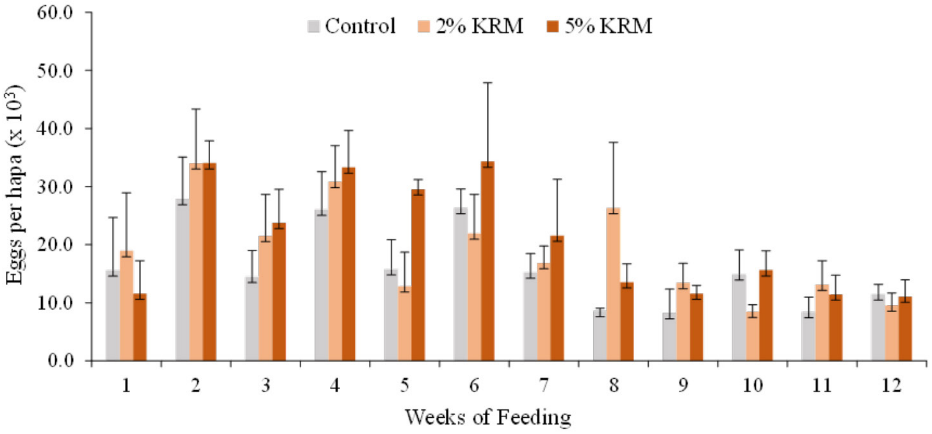
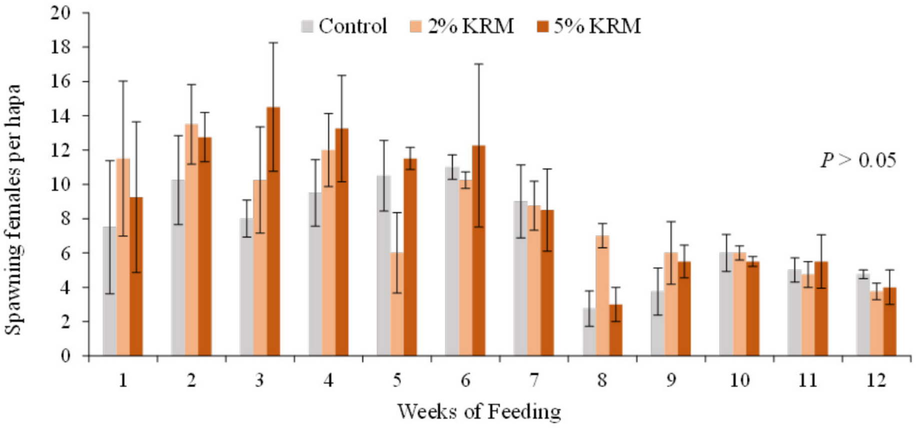
Introduction

Farmed tilapia is currently the second most farmed finfish in aquaculture and represents 5.27% of global aquaculture production. To meet these growth indexes, and to support the increasing aquaculture potential for tilapia, it is critical to provide high-quality offspring. For that, improved nutrition, feeding and management of broodstock are essential tools. Broodstock nutrition is a major factor which influences reproduction spawning frequency, egg quality and larval quality. Hence it is utmost important to obtain knowledge on the nutritional requirements of the broodstock. This could lead to the development of broodstock feeds that are nutritionally optimized, effective, and cost effective to achieve optimal yields. Krill meal (KM), obtained from Antarctic krill (*Euphausia superba*) has rich nutrition profile including phospholipids, high levels of long-chain polyunsaturated fatty acids, such as EPA and DHA, and astaxanthin, and free amino acids, nucleotides and trimethylamine N-oxide (TMAO), has been vastly demonstrated to be beneficial in enhancing growth and health of different fish species including Nile tilapia. However, so far, there are no reports on the effect of KM in enhancing the broodstock performance of tilapia. Hence, the present study was conducted to determine the effect of KRM in broodstock diets for Nile tilapia, on spawning, egg quantity and quality, and survival of larvae of broodfish Nile tilapia.





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Materials and methods

Two doses (2% and 5%) of krill meal, KRM (QRILL™ Aqua, Aker BioMarine Antarctic AS, Lysaker, Norway), were tested in the broodstock diets for Nile Tilapia, in comparison to a commercial tilapia broodstock feed produced in the USA. 12 hapas (submerged cages) were employed in this study, with 4 hapas/diet group. Fish were stocked at an industry standard 3:1 sex ratio. The feeding trial was conducted at Benchmark Genetics - Spring Genetics in Florida, USA. The fish were fed their respective test diet for 12 weeks.

Results

Over the course of 12 weeks, trends for positive effect of KRM diets were observed towards total egg production (2% KRM has 20% more total eggs than control; 5% KRM has 30% more total eggs than control), total number of spawning females (2% KRM has 13% more spawning females than control; 5% KRM has 20% more spawning females than control), and larval survival 10 days post-hatching (5% KRM has 10% more larvae than control). In addition, the proximate composition of eggs indicated higher fat content in KRM fed groups in comparison to the control group.

Conclusion

This is first pilot study demonstrating the trends of positive effects of KRM towards reproductive performance and larval survival in Nile Tilapia.

SINTEF ACE ROBOTIC LAB – AUTOMATION AND ROBOTICS TO DRIVE UP EFFICIENCY AND MINIMISE RISKS IN FISH FARMS

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Introduction

Aquaculture is an important global contributor to the production of seafood for human consumption. However, this industry is also known for a substantial HSE risks and a high frequency of work-related injuries. Consequently, increasing the automation level of high-risk operations within aquaculture could lead to economical as well as social and ethical benefits. Increased automation can contribute to improving the level of control humans have over aquaculture operations by facilitating increased use of technological solutions and unmanned underwater vehicles (UUVs) [1]. To target these challenges, the newly established SINTEF ACE-RoboticLab aims to be the pioneer for developing both basic and applied research on autonomy and robotics with the overall target being to contribute increasing efficiency and objectivity during daily operations in fish farms, and thus provide solutions that will contribute to addressing the current challenges in the aquaculture domain (Figure 1).

Materials and methods

Research on autonomy and robotic solutions in SINTEF Ocean targets the development of dedicated solutions for the aquaculture industry and thus aims to address the industry's challenges for optimal and sustainable production. Therefore, as a part of the SINTEF ACE infrastructure hub, SINTEF Ocean recently established the SINTEF ACE-RoboticLab which aims to create innovative tools and autonomous solutions which expand the ability of the aquaculture industry and improve precision and efficiency in the aquaculture segment. To accomplish the vision of the lab, we pursue projects focused on autonomous and robotised solutions applied to the aquaculture industry [2]. Our team's vision has targeted research and solutions for autonomous intelligent systems capable of long-term autonomous operations in unknown, complex and dynamically changing environments in the aquaculture industry.

One of the main goals of SINTEF ACE-RoboticLab is to introduce and develop innovative solutions that will be able to operate in any environment and in interaction with the biomass under any possible conditions, while performing demanding aquaculture operations [3]. The SINTEF ACE-RoboticLab research group addresses the challenges related to the development of autonomous technological solutions and control of systems that adapt to different situations and cope with an uncertain and dynamically changing environment. With a large focus on interdisciplinary research linking the fields of technology and biology, we perform fundamental research on the modelling of UUVs, aquaculture structures, fish behaviour and environmental disturbances, and on advanced control strategies for autonomous navigation and exploration of unmanned underwater and surface vehicles operating in dynamically changing environments.

Results

Through variety of projects, we developed methods and robotic solutions for autonomous operations in fish farms (to name few of them: CHANGE, ResiFarm, NetClean 24/7, Autosmolt2025, RACE-Fish Machine Interaction, RACE Digital Cage, SalmonInsight, Indisal, Crowdquard, CageReporter, Artifex, Bioracer, Sensordrone, MerdROV and several others). We own and use a variety of robotic systems including aerial, surface and underwater vehicles. In addition, the group contributes to the development of new, dedicated robotic systems suitable for operating in fish farms. Aquaculture Robotic Simulation Framework has also been developed in our group based on the FhSim – Simulation of Marine Operations and Systems, which features a large collection of mathematical models including ships, fishing trawl systems, aquaculture net cages, closed containment systems, fish behaviour and energetics, feeding systems, remotely operated vehicles (ROVs) [2]. We have a very close collaboration with the SINTEF ACE – Facilities and resources for Precision Fish Farming (PFF), and technology for better fish welfare to secure developing technological solutions that match the biological perspectives. Several autonomous features have been demonstrated in industrial scale fish farms (Figure 2-3).

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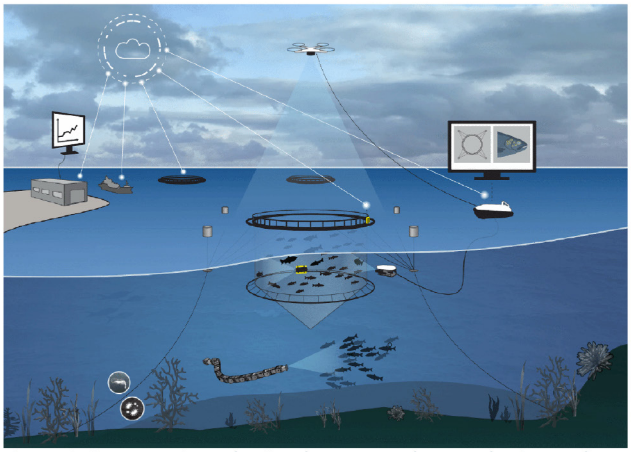

Figure 1: SINTEF ACE-RoboticLab – Aquaculture Robotics and Autonomous Systems Laboratory: Development of new knowledge and technology for optimized operations in fish farms

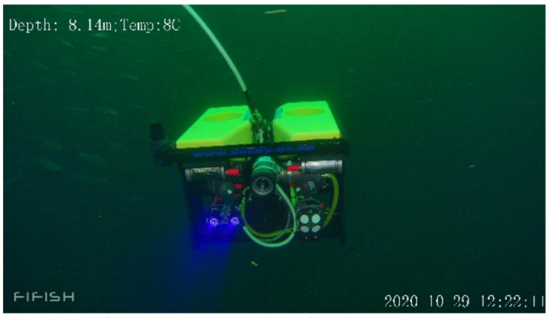

Figure 2: The ROV equipped with a laser.

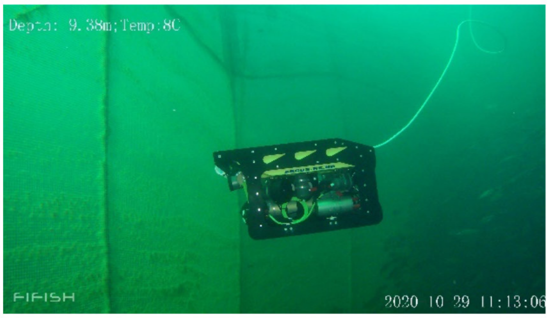

Figure 3: The ROV during net following.

Conclusion and future work

Management of sea-based fish farms typically entails manual, and often challenging, inspection operations to monitor equipment, structures and biomass, which may result in sub-optimal and costly operations, insufficient maintenance, a general lack of control in daily routines and potential high risks for welfare of personnel and fish. This implies a need for new methods and technology for operations in modern fish farms, especially when moving operations to more exposed locations with more challenging environmental conditions, and new farm designs. Therefore, the goal of establishing the SINTEF ACE – RoboticLab is to contribute to making operations in the aquaculture industry more efficient by using different types of autonomous concepts and robotic systems. We aim to develop new knowledge and technology for the underwater robotics systems of the future, where these systems must coexist and cooperate with living fish and flexible structures during operations. By incorporating biological parameters (i.e. fish behaviour) into the mathematical models, this interdisciplinary approach will provide a foundation for the resident underwater robotics systems of the future and thus enable them to operate in adaptation to live fish. This impact will be expressed both in the short term by enabling new research activities, and in the long term by resulting in innovations that help industries to improve the safety and efficiency of using robotics systems in demanding operations.

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AN AUTOMATED ANALYSIS OF THE DISTRIBUTION AND HOME RANGE OF SMALL GROUPS OF TANK-HELD ATLANTIC SALMON PARR (*Salmo salar* L.) USING COMPUTER VISION

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Introduction

Operational Welfare Indicators (OWIs) are the tools a stakeholder uses for monitoring fish welfare. A welfare indicator that has particular utility is behaviour (e.g., Dawkins, 1999), but it is often difficult to quantify, and the auditing process is usually manual and time consuming (Noble et al., 2018). The emerging digitalisation of fish welfare monitoring can provide automated tools for quantifying fish behaviour in experimental and commercial settings. This can provide stakeholders with a wider and more rapid overview of changes in fish behaviour, helping them make more informed and robust decisions in both the short- and longer-term.

The main goal of this study was to develop a deep learning framework to automatically extract and analyse a variety of behavioural traits of Atlantic salmon parr held in experimental tanks. Specifically, the first aim was i) to apply the deep learning framework DeepLabCut (DLC; Mathis et al., 2018) to identify key body parts on the fish, and ii) use this information to determine the distribution of fish and quantify its activity in relation to feeding events.

Material and Methods

Video footage used in this study was sourced from a series of experiments focusing on social network analysis of Atlantic salmon parr (*Salmo salar* L.) in 300 l plastic circular tanks, holding six fish (137.5±20.1g) at initial stocking densities of 4.1 ± 0.1 kg/m^3 (see Cañon Jones et al., 2010 for more information). A video camera (Panasonic© VWR42 with Panasonic© WV-LA4R5C3B lenses) was placed one metre above the water surface. Two videos, A (10 minutes duration) and B (20 minutes duration) recorded at 25 frames per second (fps), have been used in the study. The first video (A) documents fish in standard non-feeding conditions. The second video (B) includes six feeding events where feed was dispensed for ca. 1 second every 3 minutes by an automatic feeder.

The DLC framework was used to estimate the x and y coordinates of seven keypoints distributed along the dorsal length of the fish. Such keypoints were manually annotated over 700 video frames for model training and validation purposes. A workstation with two instances of NVIDIA GeForce RTX 3090 run on an AMD Ryzen Threadripper 3970X was used for annotation. To visualise the data, fish coordinates were plotted using heatmaps documenting activity over one-minute intervals following feed delivery (i.e., three intervals), and the home range was defined as the region holding 50% of detections in Video A. The probability mass and probability density functions were calculated using the Gaussian Kernel density estimate. The overall DLC framework used in the study is outlined in Figure 1. Heatmaps were quantified based on the proportion of key points that were detected outside the home range of the fish.

Results and discussion

The trained model successfully estimated key points of the fish with less than 1 % error and false detections. Fish distribution in the tanks, within and between feeding events is illustrated as a 3x6 matrix of heatmaps in figure 1(c). The area directly in front of the water inlet was the preferred location for fish in this tank, where fish generally held a stationary position facing the water current. Analysis reveals the fish exhibit a large foraging home range during the first feed of the day. As the number of feeding events increase, the home ranges of individual fish slowly decrease back to baseline non-feeding levels. By the fifth feeding event, home range is similar to baseline levels and fish stop feeding by the sixth feeding event. These results demonstrate that the automated analysis of home range around a feeding event can be a promising feed management tool for small groups of tank-held salmon in experimental studies. Variability in home range around a meal can also be a potential proxy indicator for both appetite and satiation.

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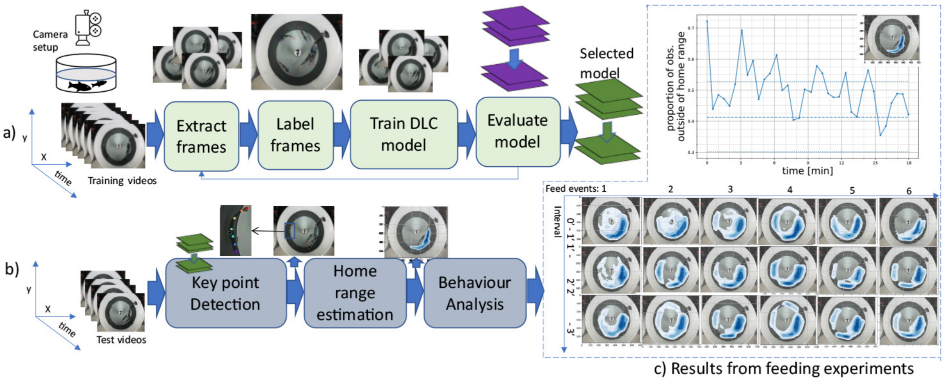

Fig. 1 An overview of the DLC framework used to analyse the home range and distribution of the fish. The first part outlines (a) the steps taken to annotate, train and develop a deep learning model to estimate and predict key body parts of the fish. The second part outlines (b) how fish swimming trajectories were used to assess fish distribution and home range within the tanks in and around a meal. The results of the study are outlined in (c).

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Acknowledgements

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FREE AMINO ACIDS MIX SUPPLEMENTATION ON DIET IMPROVES GROWTH PERFORMANCES, FEED UTILIZATION, ANTIOXIDANT AND IMMUNE RESPONSES, DIGESTIVE ENZYMES AND FATTY ACID COMPOSITION OF NILE TILAPIA FINGERLING *(Oreochromis niloticus)*

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Introduction

Mixes of free amino acids (MFAA) obtained from extensive hydrolysis of full protein chains are interesting candidates for aquaculture feeds. In addition to their specific amino acids profile, their low molecular weight ensure a fast and high level of assimilation with synergetic effects during the first development stages. Previous investigations on shrimp *(L.vannamei)* underline MFAA positive effects on zootechnical performances (Le Reste et al., 2019) and survival in case of bacteriological and viral challenges (Kersanté et al., 2021). The results presented here have been obtained from an eight-week study conducted to investigate the effects of a mix of 17 amino acids obtained from extensive hydrolysis of poultry keratin, on the growth performances, biochemical parameters, innate immune response, muscle composition, digestive enzyme activity and muscle fatty acid composition of Nile tilapia (*Oreochromis niloticus*) (Wangkahart et al., 2022).

Protocol

450 healthy juvenile Tilapia (*O. niloticus*), 4.76 ± 0.05g initial weight (IW) were purchased from Maha Sarakham province, Thailand. The fish were acclimatized to laboratory conditions in circular fiberglass tanks for 2 weeks, in the aquarium of the Division of Fisheries, Mahasarakham University. During this period, fish were fed with a commercial diet containing 32% of protein and 4% of fat (Charoen Pokphand Foods, CP, Thailand). After acclimatization, fish were allocated in 15 floating cages ($2*1.5*1.5m^3$) in five triplicates groups at a stocking density of 30 fish per cage, in an open circulatory freshwater system.

In order to meet or exceed the nutritional requirements of tilapia established by the National Research Council (2011), we formulated five isonitrogenous (32% crude protein) and isolipidic (4.2% crude lipid) diets, supplemented with MFAA.

Four different concentrations of MFAA were tested: 2.5 g/kg of feed, 0.25%, 5 g/kg of feed 0.50%, 7.5 g/kg of feed, 0.75% and 10 g/kg of feed, 1.00%, respectively MFAA0.25, MFAA0.50, MFAA0.75 and MFAA1.00. The MFAA was included into the pellet mix, before pelletizing.

Fish were fed twice a day (8am, 4pm). A daily feeding rate of 5% of the biomass was applied. It was adjusted every 2 weeks, according to fish growth. The experimentation was conducted during 8 weeks.

Results

The data underline interesting improvements of growth parameters with MFAA supplementation. A dose effect could be seen with best performances achieved for animals fed with MFAA1.00 treatment, generating significant gains on biomass evolution with final weight (FW) improved by 28.9%, weight gain (WG) by 31.5%, specific growth rate (SGR) by 8.9% and a feed conversion ratio (FCR) reduction of 24% after 8 weeks ($P<0.05$).

Interestingly, growth performances are also correlated with modifications in body composition indices and feed utilisation. These parameters clearly underlined better performances for all MFAA treatments ($P<0.05$), with significant gains, related to a dosage effect of MFAA, on protein efficiency ratio (PER) improved by 29.2%, carcass yield by 9.3% and a reduction of the viscerosomatic index (VSI) of 19.5% for MFAA1.00 ($P<0.05$).

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Fillet composition analysis underlined significant influence of MFAA on the fillet lipid content (+88% with MFAA0.75). Thirty-seven different fatty acids were exanimated in the muscle tissues of the fish following the different diet treatments. Most of the analysed individual monounsaturated fatty acids (MUFA) such as palmitoleic acid (C16:1), oleic acid (C18:1 n9c), and erucic acid (C22:1 n9), as well as the total amount of MUFA, were found to be higher in the muscle from fish fed any MFAA diet compared to the control group ($P<0.05$). Interestingly, linoleic acid (C18:2 n6c) and α–linolenic acid (C18:3 n3) levels were higher in the fish fed MFAA0.75 and MFAA1.00 diets ($P<0.05$).

In relation with these observations, the fillet color measured on ventral and dorsal regions was also significantly influenced with higher value of L, expressing the lightness and white color of the fillet, respectively +13.7% and +7.2% for the L value of the fillet ventral and dorsal regions with MFAA0.75 ($P<0.05$).

Digestive enzyme activies (amylase, lipase and proteinase) were significantly increased with MFAA0.75 and MFAA1.00 treatments, respectively from 34.9%, 10.4% and +39.9%, in comparison with the negative control ($P<0.05$).

Discussion

This study underlined some particularly interesting effects of MFAA when applied on Nile tilapia fingerling feed. Firstly, regarding growth parameters with positive effects on biomass and feed utilization. Interestingly, we underline strong improvements of VSI, fillet composition and fillet color. We can hypothesize a relation between these observations and a better assimilation of the feed due to an optimisation of the energy by the digestive system. In addition, we can hypothesize that the more pronounced white color of the fillet is probably in relation with a reduction of fat oxidation.

Interestingly, we noted a reduction of the VSI, potentially linked with a higher amylase activity generating a better utilization of the polysaccharides. This parameter probably induce a lower fat deposition around the viscera and a higher lipid transfer to the fillet. The results also revealed higher contents of important fatty acids such as oleic acid (C18:1 n9c), linoleic acid (C18:2 n6c) and α–linolenic acid (C18:3 n3) in fish fed with MFAA.
The strong effects of MFAA on digestive enzymatic parameters with positive impact on body condition indices and fillet quality open new development possibilities to improve fish feed utilization and farming performances.

This new field of application confirms their interest as a sustainable protein source converted into an efficient functional ingredient for fish nutrition.

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DEVELOPMENT OF A POLYMERASE CHAIN REACTION (PCR) AND DIG-LABELLED *IN SITU* HYBRIDISATION FOR THE DETECTION OF *Vibrio aestuarianus* AND OTHER *Vibrio* BACTERIA

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Introduction

Vibrio bacteria pose a significant threat to fish and shellfish aquaculture with *Vibrio*-associated mortality episodes impacting stocks in aquaculture settings globally. *Vibrio aestuarianus* is especially problematic in Pacific oyster *Crassostrea gigas* culture. Diagnostic tools currently used to detect or confirm *V. aestuarianus* are real-time quantitative (q)PCR and fluorescent *in situ* hybridisation (FISH). These tools are expensive and can demand highly specialised equipment. This study aimed to develop a novel generic PCR and DIG-labelled *in situ* hybridisation (ISH) for the detection and confirmation of *Vibrio* bacteria. These tools allow for blanket screening of hosts and environmental samples, as well as the microscopic localisation of bacterial infections. Both diagnostic methods were developed for use synchronously with other methods that are specific to certain *Vibrio* spp.

Material and methods

New primers (VibF3/VibR3) were constructed to amplify a partial sequence from the 16S ribosomal RNA gene region which is common to all *Vibrio* spp. with a resulting PCR product of 286 bp. These novel F3/R3 primers were then used to create a DIG-labelled probe for use in a newly designed *in situ* hybridisation technique. Confirmatory positive (plasmid DNA) and negative (dH_2O) controls were used in the PCR and ISH method design. Diagnostic findings were compared to a specific qPCR for *V. aestuarianus*. Direct Sangar sequencing of PCR products, Blastn analysis and Clustal Omega alignments were used to confirm *Vibrio* sp. Identification and assess similarity, histology was used to examine the pathology of *Vibrio* infections in oyster tissues.

Results

The PCR was shown to be sensitive compared to established protocols. A positive ISH signal was observed in qPCR and PCR positive oysters and no signal was detected in uninfected individuals. Pathologies observed in *C. gigas* tissues were similar to those described in other studies. These techniques will increase the diagnostic arsenal against this globally significant genus of bacteria.

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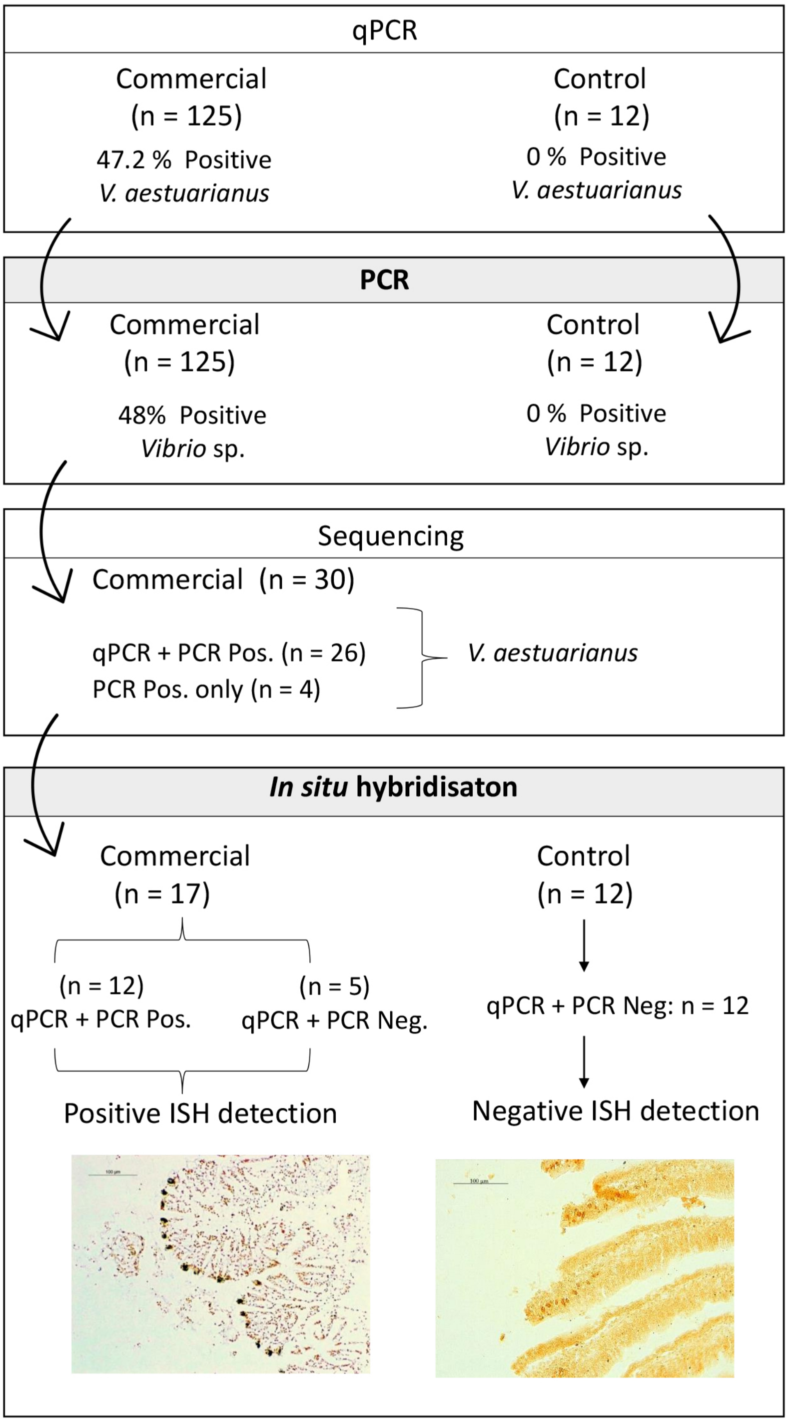

Figure 1. Graphical abstract describing the sampling design and methodology used to compare and validate new diagnostic tools (PCR and *in situ* hybridisation) Pos. = Positive for infection, Neg. = Negative/Uninfected.

GENETIC VARIATION IN GROWTH IN ATLANTIC SALMON WHEN FED CONVENTIONAL AND INNOVATIVE DIETS

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Introduction

Genotype-by-environment interaction (GxE) is a measure of the magnitude of re-ranking of genotypes across environments. Significant GxE creates discrepancy between the expected and realized performance when the selected genetic material from the breeding nucleus is reared in divergent environmental conditions in commercial aquaculture. Estimates of GxE are lacking for many economically important traits (Sae-Lim *et al*., 2015) and need to be assessed to enable optimization of breeding programs towards development of robust genetic material for future conditions. Use of alternative raw materials in fish feed formulation is prompted by many regulatory, climatic and societal factors to guarantee future sustainable European aquaculture (Skiba *et al*., 2015). This brings upon a need to assess whether there is significant genotype-by-feed interaction; breeding companies are dependent on the assessment whether their current genetic material can express the production potential when alternative feed sources are utilized. The objective of this study was to estimate genetic parameters of growth in Atlantic salmon when fed with conventional and innovative diets.

Material and methods

Individually tagged Atlantic salmon from 75 full-sib families (Benchmark Genetics Norway) were randomly assigned in four parallel 5m x 5m cages at Gildeskål Research station (Inndyr, Norway), where two of the cages were fed with conventional marine-based diet (N=1993) and two with innovative diet (N=1960). Both feed types were formulated and produced at Aquafeed Technology Center (Nofima AS, Bergen, Norway) and were isoenergetic. In the innovative diet, 50% of the marine oil was replaced by algae and 75% of the fishmeal by insect meal. Additionally, innovative diet had no soya.

In addition to body weight (EWT), $TGC = \frac{\sqrt[3]{EWT} - \sqrt[3]{TAGWT}}{day\ degrees} * 1000$ (thermal growth coefficient) and $ADG = \frac{EWT - TAGWT}{duration\ of\ experiment}$ (average daily gain) were calculated and used as phenotypes. Multivariate model treating phenotypes among diets as different traits was fitted to estimate the genetic parameters using restricted maximum likelihood in BLUPF90 (Misztal *et al*., 2018). Genomic relationship matrix was used in the estimation.

Results and discussion

The fish from the conventional diet were at the end of the trial significantly larger (1581 g) than those fed with innovative diet (1372 g). The mortality rate in groups fed with the innovative diet was 32.2% whereas in cages fed with conventional diet it was 19.4% (overall 25.8%). The frequency of deformities (6%) and wounding (0.6%) were low, and only three individuals were registered as sexually mature. During the summer 2020 high mortalities were registered and the disease heart and skeletal muscle inflammation (HSMI) was verified causing 6% mortality independent on the diet.

Families expressed large phenotypic variation in EWT in both diets (CV=0.28) (Figure 1). Only two families had higher average growth with the innovative diet than with conventional diet. The difference in EWT family average varied from -5.8 to 32.8%. Phenotypic rank correlation for growth phenotype family means varied between 0.81 and 0.85, indicating only low re-ranking of families between the diets.

Estimates of heritability for growth traits were high: 0.41-0.45, and genetic correlations between growth traits among diets were very high (0.96-1.00), indicating no re-ranking of genotypes across diets (Table 1). Heritability for survival was low very low for both innovative (0.03) and conventional diet (0.11). Genetic correlation for survival indicated moderate re-ranking of genotypes across diets: r_g=0.68.

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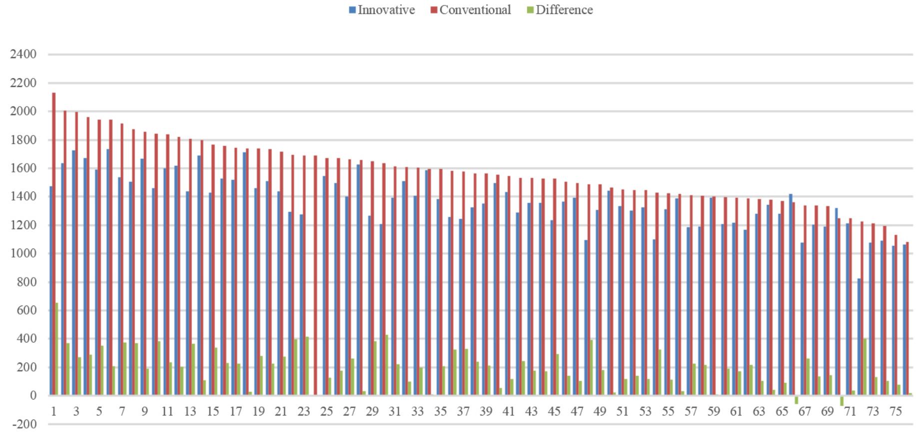

Figure 1. Phenotypic family means (and their difference) for EWT for innovative and conventional diets.

	EWT_fut	**EWT_conv**
EWT_fut	**0.44 ± 0.00**	
EWT_conv	1.00	**0.45 ± 0.00**
	TGC_fut	**TGC_conv**
TGC_fut	**0.41 ± 0.03**	
TGC_conf	0.96 ±0.01	**0.44 ± 0.03**
	ADG_fut	**ADG_conv**
ADG_fut	**0.44 ± 0.03**	
ADG_conv	1.0 ±0.01	**0.45 ± 0.03**

Table 1. Estimates of heritability and genetic correlations for growth traits across the diets. Estimates of heritability in sub-diagonals (**bold**).

Our study showed that growth in Atlantic salmon is impaired when fed with innovative feed where large proportions of marine ingredients are replaced by algae and insect meal. We did not detect genotype-by-feed interaction in growth in Atlantic salmon during this limited growth period from average body weight of 135 g until ~1.5 kg, whereas moderate genotype-by-feed interaction was detected for general survival.

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EFFECT OF VITAMIN C (L-ASCORBIC ACID) SUPPLEMENTED FEEDS ON THE GROWTH AND NUTRIENT PROFILE OF BULLSEYE SNAKEHEAD *Channa marulius*

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This study was conducted to determine the impact of dietary vitamin C (L-ascorbic acid) on the growth, proximate composition, amino acid and fatty acids profile in bullseye snakehead *Channa marulius* (100 g initial body weight body weight) reared in cages for three months. Fish were fed a 40% crude protein diet supplemented with various levels (0, 75, 150, and 300 mg kg^{-1}) of vitamin C in triplicates. After the feeding trial, fish fed 300 mg kg^{-1} dietary ascorbic acid shown significantly ($P<0.05$) better growth including net weight gain (104.33 g), FCR (1.67), and SGR (0.74 %) than those fed 150, 75, and 0 mg kg^{-1} (vitamin C free diet) respectively. Proximate composition of the fish was significantly influenced by the dietary treatments ($P<0.05$). The highest protein content 19.61% and lowest moisture content 72.65 % were found in T1 (75 mg kg^{-1}). Dietary vitamin C levels had also a significant effect ($P<0.05$) on the methionine, threonine, leucine, histidine, lysine, and glutamic acid of fish muscle. There was no significant difference observed in the fatty acid profile ($P>0.05$) of fish. Conclusively, including 300 mgkg^{-1} of vitamin C in the diet for snakehead in artificial pellet feed improved fish growth without affecting nutrients profile.

THE EFFECT OF DIFFERENT LIVE FEED ORGANISMS ON LARVAL LUMPFISH (*Cyclopterus lumpus*)

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Introduction

Lumpfish (*Cyclopterus lumpus*) is a cold-water species much used as a cleaner fish to combat the sea lice problem in sea-based salmon farming. The commercial production has increased to around 35 million juveniles in 2020 (Norwegian Directorate of Fisheries, 2022). Lumpfish larvae are commonly fed formulated feeds from 2 to 5 days post hatch (dph). The nutritional requirements of the lumpfish larvae are largely unknown, and the production often results in variable growth, survival and delousing efficiency. Suboptimal nutrition during early life history in any animal may have lasting effects (Winick and Noble 1966), with possible suboptimal health and welfare for later life stages. Wild lumpfish larvae and juveniles mainly feed on crustaceans (Ingólfsson and Kristjánsson, 2002), and copepod and cirripedia early life stages may have the potential to improve their growth and survival. If lumpfish larvae are fed *Artemia*, they grow better than if they are fed formulated diets (Brown, 1986; Kjørsvik, unpublished); but high mortalities can occur during weaning from *Artemia* to formulated diets at 25 – 30 dph (Powell et al., 2018).

Our aim was to evaluate the effects of different feed types (formulated diet, enriched *Artemia*, cryopreserved cirripedia, and cultivated copepods) and different feeding regimes on growth and survival. We evaluated several developmental parameters and functional responses in the larvae such as bone ossification, skeletal muscle and liver development, and molecular as well as microbial and biochemical parameters.

Materials and methods

In a 35-day start feeding experiment (34 ppt salinity, 10 °C) with lumpfish larvae, the larvae received different feeds from 2 - 21 days post hatch (dph). Five different treatments were tested: (1) Control: formulated diet (Gemma micro 150 and 300); (2) enriched *Artemia* nauplii; (3) cirripedia (cryopreserved cirripeds from Planktonic AS); (4) *Acartia tonsa* nauplii and copepodites (2-9 dph, from CFEED AS) and weaned to formulated diet GEMMA micro 150 ("Cop/FD"), and (5) *A. tonsa* nauplii and copepodites and weaned to cirripedis ("Cop/Cir"). All larvae were weaned to a common formulated diet (Gemma micro-300) from day 21 – 35 dph, with a period of co-feeding from day 21 – 25 post hatching.

Results

Growth rates in larvae fed *Artemia* were significantly higher than for all other groups during the first 30 dph, with larvae fed copepods and formulated diet showing the lowest growth (Fig. 1). The larvae fed cirripedia had a slow growth during the live feed period but demonstrated a much higher growth rate after weaning to the formulated diet. At the end of the experiment (34 dph), no difference in SL and DW growth were found between larvae fed *Artemia* and cirripedia, whereas larvae from all other groups were significantly smaller.

Histology showed that the degree of liver vacuolization was a good marker for nutritional condition in lumpfish larvae, and no dietary effects on muscle development were observed in relation to body size. Larvae fed *Artemia* had significantly higher fin ossification scores than larvae from the other groups in relation to both size and age, followed by the Cirripedia- and Cop/Cir- larvae.

Microbiological diversity (*alpha* and *beta* diversity) showed significantly higher microbial diversity in larval samples compared to water samples. The total gene expression profile indicated very similar larval expression profile in all treatments at 10 dph, with more diverse responses between the larval groups at later stages. Lipid analyses are still being processed.

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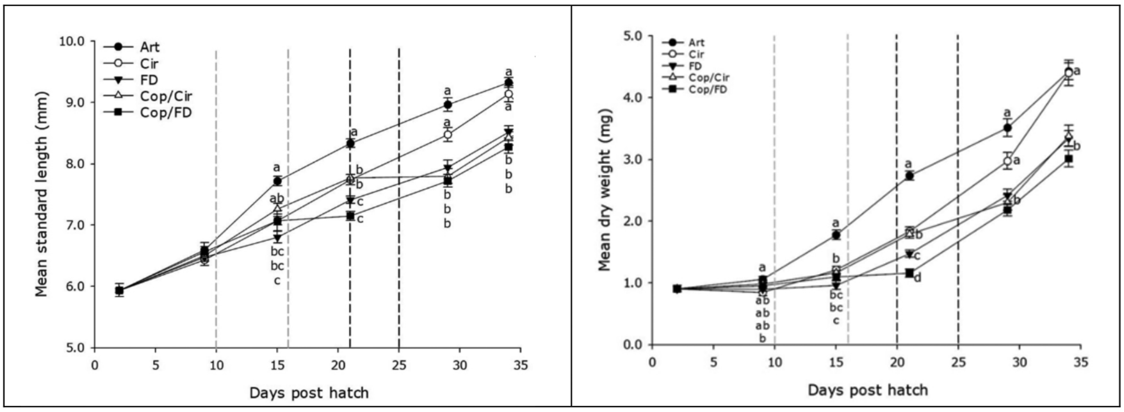

Figure 1. Lumpfish larval growth in relation to start-feeding regimes. Mean standard length (left) and mean dry weight (right) of lumpfish 2-34 dph. Grey dashed lines indicate weaning from A. tonsa *to either formulated diet for the Cop/FD group, and to cirripeds for the Cop/Cirr group. Black dashed lines indicate weaning to formulated diet for all groups. Significant differences (p<0.05) between treatments at a given dph are indicated by different letters. Error bars indicate ± standard error (SE).*

Discussion and conclusions

In our experiment, using enriched *Artemia* nauplii caused early fast growth and good development, whereas feeding with cryopreserved cirripedes lead to higher growth rates in the later stages of the experiment and during weaning onto formulated feeds. These two prey items also resulted in the highest survival and the largest larvae by the end of the experiment. Startfeeding with copepod nauplii or formulated diet both resulted in the overall slowest growth, and the earliest poor nutritional status during the earliest development might have had a long-lasting effect with poor growth and development throughout the whole experiment. The results will be discussed in relation to our mapping of biochemical, histological, molecular and microbial parameters.

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BUBBLES AS A FEEDING PREDICTOR IMPROVES THE WELFARE OF FARMED RAINBOW TROUT

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Introduction

Environmental enrichment is now a well-established practice to improve fish housing conditions for a positive state of welfare, both mental and physical (Arechavala-Lopez et al., 2021). Occupational enrichment emerges as a promising strategy for improving the welfare of farmed animals. This form of enrichment aims to stimulate cognitive abilities of animals by providing them with more opportunities to interact with and control their environment. However, occupational enrichment, and its effects on fish welfare indicators, remains a recent and understudied topic in fish production. Predictability of salient daily events, and in particular predictability of feeding, is currently one of the most studied occupational enrichment strategies and can take several forms (Bassett & Buchanan-Smith, 2007). In fish, while temporal predictability (based on the time of day) of feeding has been widely investigated (Cañon Jones et al., 2012; Chapman et al., 2010; Holley et al., 2014; Sánchez et al., 2009; Ferrari et al., 2016)but rather as an adaptive benefit. This variability comes from consistent differences in behavioral and physiological responses among individuals to a changing/challenging environment, named \"coping style\", \"temperament\" or \"personality\". Many studies have described how to characterize personality traits and how to assess their consistency over time and between different contexts; however, little is known about the environmental factors shaping personality development. Because contrasting personalities are maintained with evolution, this lead to the widespread assumption that genes play a predominant role in personality. In many cases, personality traits are however also likely to be determined by individual experience, which is probably at least as important as genetics in shaping personality. The aim of this study was to assess how environmental variability (herein food predictability, signalled predictability (based on a signal, such as light or sound) has received little attention (Cerqueira et al., 2017; Galhardo et al., 2011). These studies result in differing effects on fish welfare, depending on the type of predictability used and the ecology of the species. The present study aimed to determine which feeding predictability would be most appropriate for rainbow trout, the main continental farmed fish in Europe, and what the consequences might be for their welfare.

Materials and Methods

We tested four feeding predictability conditions for two weeks: temporal (based on time of day), signalled (based on bubble diffusion), temporal + signalled (based on time and bubble diffusion), and unpredictable (random feeding times). Behavioural and zootechnical outcomes recorded were swimming activity, aggressive and stereotypic behaviours, emotional reactivity, and growth.

Results

Our results revealed that rainbow trout can predict five daily feedings with time as the only predictor as early as two weeks of conditioning. However, this predictor alone also increased aggressive and stereotypic behaviours, suggesting that the use of time as the sole predictor of feedings in husbandry practices may be detrimental to fish welfare. The combination of temporal and signalled predictability elicited the highest conditioned response and the level of pre-feeding aggression and stereotypies tended to be lower than for temporal predictability alone. Rainbow trout were also successfully conditioned to use bubbles as the sole predictor of feeding, and this condition resulted in fewer pre-feeding agonistic and stereotypic behaviours than in the temporal predictability condition. Interestingly, we also found that bubbles were highly attractive regardless of the condition considered.

Conclusions

The use of bubbles as a feeding predictor could be an interesting approach to improve rainbow trout welfare in farms, by acting as both an occupational and physical enrichment.

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Acknowledgements

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ALTERNATIVE WAY OF EXPLOITING THE NON-NATIVE FISH OF THE GENUS *Lagocephalus*. DEACTIVATION OF TTX AND FISHMEAL PRODUCTION FOR USE IN MEDITERRANEAN AQUACULTURE

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Introduction

In recent years, a massive invasion of non-native marine species has been observed in the Mediterranean Sea. Particularly, among 17,000 existent species, more than 1,000 are non-native species of which, more than 300 have been recorded in Greece. A large number of those non-native species are considered invasive and have caused significant ecological problems. Some of those are the displacement of native species, genetic drift, structural change of native communities, food web alterations, and others. In addition, their presence has damaged local economies, as fishery and tourism are affected.

A characteristic example of a Lessepsian migrant is the genus *Lagocephalus*. *Lagocephalus* belongs to the family of Tetraodontidae. *Lagocephalus* sp. which is not a target for inshore fishing, due to its toxicity and related marketing restrictions (Katikou et al., 2009). In addition, *Lagocephelus* sp. is less likely to compete or be hunted by other species. All the above factors, as well as the fact that it can be caught by almost all types of fishing gear, lead to its excessive fishing as a bycatch (Nader et al., 2012).

The potent exogenous neurotoxin, TTX, is found in the *Lagocephalus* sp. tissues. TTX is produced by marine bacteria, such as *Vibrio alfinolyticus*. The concentration of this neurotoxin depends on the developmental stage of the fish. Some human case reports demonstrated serious effects at a dose of 0.2 mg, corresponding to 4 μg/kg body weight (EFSA, 2017).

Lagocephalus sp. has already adapted to the habits of the Mediterranean Sea and is causing long-term damage, by disrupting the ecology of its living areas, and short-term damage, by destroying fishing gear and especially those of small-scale coastal fishery. Therefore, it is imperative to find ways to exploit this new fish species, which will augment the revenue of the fishery sector as well as the aquaculture sector. The present study focuses on the use of *Lagocephalus* sp. for fish meal production, as the fundamental ingredient of fish food. Thus, the deactivation of TTX from fish tissues is essential.

Materials and methods

In total 39 individuals of *Lagocephalus sceleratus* were collected from the Aegean Sea and were freshly transferred to the labs of HCMR, where a somatometric analysis took place. Subsequently, the fish were grounded and their sex was determined macroscopically by visual inspection of gonads. The fishmeal was obtained after cooking, press drying the fresh grounded fish and milling successively. In the stage of press drying, most of the moisture and some or all of the oil were removed (stick water, SW). After press drying, the wet meal (WM) was left overnight in an air oven to dry and the final dry meal (DM) was collected the following day. Finally, fishmeal (FM) was treated at different temperatures (ranging from 160 to 510°C) and exposure times (ranging from 10 secs to 40 min). TTX heat inactivation was then analyzed by TTX ELISA (EuroProxima, 5191TTX) and liquid chromatography in combination with sequential mass spectrometry (LC-MS / MS) (Christidis et al., 2021). The statistical analysis was done by the GraphPad Prism v. 8.4.2. software.

Results and Discussion

Total length (TL) ranged from 21.5 to 72cm, total weight from 100 to 4300g. From the 39 *L. sceleratus* samples, 19 were males and 13 females. The initial processing of the samples at three different exposure times (30-60-90 min at 80°C) did not affect the TTX concentration. Furthermore, almost identical concentrations of stick-water and WM (the two products resulting after the press stage) were observed, revealing that approximately 50% of the TTX is removed from the sample at this stage. Nevertheless, the concentration in the samples still remained at a high level. According to both TTX detection methods (LC-MS/MS and ELISA), heat treatment of the samples at 200°C for 5 min, 210°C for 10 min, and 225°C for 1 min resulted in complete inactivation of the toxin (below the detection limit, Figure 1). Specifically, the ELISA method

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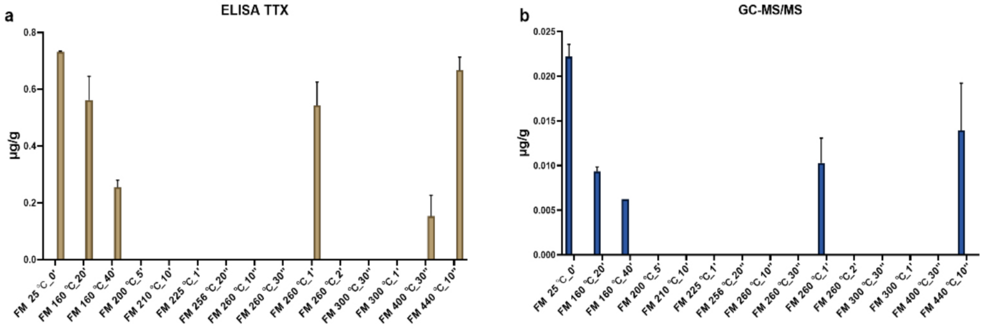

Figure 1: Deactivation of TTX (μg /g) after heat treatment of fishmeal (FM: Fish Meal) at different times and temperatures. **a.** Biochemical ELISA method and **b.** Liquid chromatography method.

showed that treatment at 210°C for 20 min and at 160°C for 40 min, reduced the TTX concentration from 3.4 μg/g to 0.5 μg/g and 0.2 μg/g, respectively. While, very low concentrations were detected at 160°C for 40 min, 260°C for 2 min and 400°C for 30 sec. Analysis of the samples by liquid chromatography showed excellent results (below the detection limit) at 160°C for 40 min and 400°C for 30 sec. Despite the differences in the order of magnitude between the two methods, it appears that both ELISA and chromatography come to common conclusions (Figure 1).

Both temperature and exposure time are equally important factors. Particularly noteworthy is the fact that concentrations below the detection limit were observed at temperature values even 100°C lower compared to previous studies. In conclusion, the present study showed the successful deactivation of TTX in fishmeal produced exclusively by *Lagocephalus*, through its exposure to specific temperature-time conditions, to utilize it for the production of fishmeal. The produced innovative raw material will be evaluated for its nutritional value as the main ingredient for the nutrition, health, and quality of the final product of cultured Mediterranean species.

Acknowledgements

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PHYSIOLOGICAL CHANGES OBSERVED IN FARMED ATLANTIC SALMON (*Salmo salar* L.) WITH NEPHROCALCINOSIS

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Introduction

The salmon industry is one of the most important industries in rural Norway and the industry is known for its innovation and use of new technologies, but welfare of farmed salmon is becoming a growing concern (Sommerset et al., 2020). Nephrocalcinosis is one of the challenges in Atlantic salmon, described as mineral deposits in the kidney. Although the prevalence of nephrocalcinosis among wild fish is unknown it is likely that the occurrence in farmed salmon is related to the intensive conditions in aquaculture (Applegate et al., 2016; Béland et al., 2020; Bjerknes et al., 1994; Klosterhoff et al., 2015; Lewisch et al., 2013). The objective of this study was to determine the prevalence and severity of nephrocalcinosis in farmed Atlantic salmon in Mid-Norway, and physiological changes related to the disease.

Material and methods

A total of 810 farmed Atlantic salmon were sampled from 14 fish groups. The fish was euthanized with a tranquilizer overdose and the following were collected; plasma, mid-kidney tissue and mineral deposits when visible. Histopathology was performed on the mid-kidney tissue and nephrocalcinosis was defined as presence of amorphous, basophilic deposits in tubules, collecting ducts and excretory ducts. The mineral deposits were analyzed using by attenuated total reflection Fourier transform infrared spectroscopy and the plasma parameters were measured with an automated dry chemistry analyzer.

Results

Nephrocalcinosis was observed in all the nurseries in this survey, even though the prevalence varied greatly. More than half of the individuals with nephrocalcinosis had mild changes in the kidney tissues. The total proportion of fish with nephrocalcinosis was 45% in the nurseries. Among fish that were sampled one month after transfer to sea, the prevalence of nephrocalcinosis had decreased to 18%.

Amorphous carbonate apatite (amCAP) was the most prevalent mineral. Struvite, brushite, whitlockite and newberyite were found in different combinations together with amCAP. A considerable proportion of fish affected by nephrocalcinosis showed plasma chemistry that differed from unaffected fish in several of the analyzed parameters, with elevated calcium and magnesium being the most predominant changes.

Discussion

The majority of salmon affected by nephrocalcinosis displayed mild changes in the kidney. The mineral deposits were mainly identified as amCAP. amCAP indicates rapid precipitation. The formation of carbonate apatite (CAP) begins at pH ≥ 6.8, with an increasing ability to aggregate with increasing pH (Olszynski et al., 2015). The pH of the urine was not measured in this study, but Roy and Lall (2004) determined that normal values for urine pH in Atlantic salmon, are around 7.5. Considering that amCAP and CAP have similar chemical properties, the normal high urine pH of salmon may predispose it for calcium phosphate precipitation in the kidney, but the underlying mechanisms of nephrocalcinosis in fish not known.

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The proportion of fish with nephrocalcinosis decreased after transfer to sea in fish with mild forms. This may indicate that mild changes in the kidney are reversible after the transfer to sea. Our data shows that fish with mild changes display elevated calcium, phosphate, AST, lactate and glucose concentrations in plasma, which are all signs of decreased welfare. Fish that were affected by moderate and severe forms of nephrocalcinosis exhibited much higher concentrations of magnesium in their plasma as well. The primary site of magnesium excretion in fish is the kidney and elevated concentration of plasma magnesium has previously been linked to kidney damage (Nieves-Puigdoller et al., 2007; Singh et al., 2002). This study revealed that plasma chemistry analyses is not adequate to diagnose nephrocalcinosis. Normal blood chemistry values do not necessarily indicate absence of nephrocalcinosis, since a notable proportion of the fish with nephrocalcinosis displayed plasma chemistry values within the normal intervals. On the contrary plasma chemistry values can be used as preliminary diagnosis for the disease, as changes in calcium, magnesium, glucose and AST were observed in a pronounced part of the fish affected by nephrocalcinosis.

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PROSEAWEED – LEARNING FROM INTERNATIONAL EXPERIENCES

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Introduction

In recent years, there has been great interest in seaweed farming in Europe for several reasons. Seaweeds are seen as a relevant source of biomass, such as food, feed, materials, biofuels, fertilisers and as a gelling agent and provide positive environmental externalities such as mitigating ocean acidification and maintaining ecosystem services such as generating habitat (Duarte et al., 2020; Duarte, Wu, Xiao, Bruhn, & Krause-Jensen, 2017; Theuerkauf et al., 2019; Xiao et al., 2021).

The European algae market so far represents a marginal portion of global seaweed production, while Asian suppliers account for around 87% of global supply (Vincent, Stanley, & Ring, 2020). Projects such as PROSEAWEED provided options for increasing the sustainable production and sustainable harvesting of algae culture in Europe with the aim to implement a successful large-scale farming, reducing transport costs and increasing the local seaweed industry and its attractiveness on the market.

Problem definition

There are currently no standardized farming methods or cultivation structures, perfectly domesticated seaweed species, or a guidebook for the ideal farming site. The risk seaweed cultivators are facing, such as weather conditions, losing one whole harvest due to unpredictable environmental hazards, or not being able to sell at a profitable price, can hinder the development of the market.

Objective

As part of PROSEAWEED, it was looked into what can be learnt from successful European seaweed farms, and how can that help to improve the business case of other new farms, for instance, in the Dutch North Sea and even large-scale seaweed farming.

Methodology

We conducted a literature research, reviewing publications on the economics of seaweed cultivation in Europe. The quantitative data was very limited and since every farm has their own approach to cultivating and harvesting, comparisons were difficult to make. We then focused on qualitative data, compiling factors that could lead a European seaweed farm to success and discussed them in detail with three successful seaweed companies.

Intended output

Learning from each other in such a young industry is key. The proposed presentation would like to display our findings on success factors for European seaweed farms findings and allow for further knowledge exchange from other seaweed farmers and even more important from other more established aquacultural industries.

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THE EFFECT OF A GENETICALLY IMPROVED COMMON CARP STOCK ON THE PRODUCTIVITY OF POND ECOSYSTEM FROM FISH FARMER POINT OF VIEW: IMPLICATION FOR SELECTIVE BREEDING IN NATURE-CLOSE CONDITIONS

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Introduction

Selective breeding (SB) is generally a very effective way for the genetic improvement of important quantitative traits in fish (Gjedrem et al., 2012). However, in most cases the SB is practiced in aquacultures with a higher level of man-managed conditions, minimally concerning the feeding of fish. Little is known about performance of genetically improved stocks in nature-close pond management of the Central Europe. Here, ponds resemble shallow natural lakes or wetlands more than man-made water works. The pond management is based on the utilization of natural food developing in ponds by fish and it is often restricted by a local legislation. The best economic model is when the growth of fish is ensured from 50 % by natural food and 50 % by a supplemental feeding. Each pond is unique water ecosystem with productivity influenced by local conditions. There is a question if genetically improved carp stocks can better utilize the pond ecosystem productivity and increase the fish mass yield (MY) per a unit of water area. Otherwise, the SB in such management had very limited applicability. This study was done to answer the open question.

Materials and methods

For the study, one genetical group of carp with lower performance (M2) and the other one with higher performance (M2 x AL) were selected. These two groups of mirror scaliness phenotype were repeatedly reared and monitored under standard pond management (water area of ponds was 0.13 – 14.3 ha) of a Czech fish farm for three years (2019-2021). Altogether, there were 61 ponds monitored, 24 of them for age category (AC) C_0-C_1, and 37 of them for AC C_1-C_2. With each stock, a control group (CG) of scaly phenotype was reared to evaluate the status of pond conditions and to correct obtained data of the two mirror stocks. The CG was of the same origin in all ponds. It was assumed that differences in the performance of the CG among ponds are given by different rearing conditions. Ponds with CG survival less than 5 % for AC C_0-C_1 and less than 40 % for AC C_1-C_2 were discarded out of the study as representing non-standard conditions with unexpected biases. Thus, the statistical analysis concerned 21 ponds with M2 and 19 ponds with M2 x AL mirror group. The data for mean weight and survival of the mirror stocks were corrected according to Linhart et al. (2002). The MY of mirror stocks per ha of pond water area was expressed as a rate corresponding to MY of the CG of the same count as the mirror group. The data were analysed for each AC and in total using several statistical approaches at significance level of $\boldsymbol{\alpha = 0.05}$, namely one-way ANOVA (in case of variance homogeneity for corrected data), non-parametric Mann-Whitney U test (in all cases) and ANCOVA (for mean growth yield and survival) with appropriate value of the control group in the same pond as covariate (Kocour et al., 2005).

Results

It was shown that carp group with expected higher performance (M2 x AL) displayed indeed better performance and MY per water area of pond within each AC as well as in total. Nevertheless, despite differences reaching up to 71 % (in TCY), most of them were insignificant no matter on statistical method used (Table 1). Still, standardized carp MY of M2 x AL group was statistically higher compared to M2 group (Table 1).

Discussion and conclusion

The productivity of ponds in Czechia is influenced by various year-to-year fluctuating factors (Kajgrov**á** et al., 2020). It may be seen on high S.D. values of parameters shown in Table 1. Huge variation among ponds very likely affected the significance of differences observed between M2 and M2 x AL groups in statistical analyses. Still, it was shown that CG M2 x AL reached higher standardized MY per ha of pond water area by 22%. The increased mass of fish resulted from better survival as well as the growth of M2 x AL. It may be suggested that the importance of these two traits on the final mass of fish will vary according to the power they will have in the ecosystem. Thus, it may be supposed that fish with genetically improved growth will use more of the pond productivity, and it will result in an increase of fish MY per ha of a pond water area. As seen from RFC, the increased MY did not change the rate of natural and supplemental food utilized for fish growth.

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Table 1. Performance and yields (mean ± S.D.) of two different mirror groups of common carp in ponds. **Corr. S** – corrected survival, **Corr. WY** – corrected weight yield, **Hyp. CY** – hypothetical carp mass yield (MY), **TCY** – Observed total carp MY, **Stand. CY** – standardized carp MY of mirror carp to MY of the control group, **RFC** – relative feeding coefficient. Values with different letter superscript ($^{a.b}$) are significantly different by one-way ANOVA, * - value is significantly higher according to the Mann-Whitney U test.

Mirror group	Corr. S (%)	Corr. WY (g)	Hyp. CY (kg) * 1 ha	TCY (kg) * 1 ha	Stand. CY (kg) * 1 ha	RFC
Age category $C_0 – C_1$						
M2	11.5±14.7	12.6±4.5	249±346.1	257±139.2	0.70±0.317	2.2±1.15
M2 x AL	17.4±13.0	14.7±2.3	373±303.9	440±399.7	0.84±0.173	0.9±0.62
Age category $C_1 – C_2$						
M2	43.9±24.0	246±49.0	452±304.1	549±343.4	0.65±0.209	2.4±0.94
M2 x AL	56.4±33.0	267±50.6	628±480.7	563±459.0	0.81±0.221	2.2±0.96
Both age categories in total						
M2	31.5±26.1	157±122.1	375±328.1	438±313.9	0.67±0.249^{a}	2.3±0.96
M2 x AL	42.0±33.1	175±131.1	534±433.5	518±430.9	0.82±0.201^{b*}	1.8±1.03

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PHARMACOKINETIC PROPERTIES OF IN-FEED ADMINISTERED PRAZIQUANTEL IN GILTHEAD SEABREAM, *Sparus aurata*

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Introduction

Praziquantel (PZQ) is an anthelmintic drug discovered in 1970s. As antiparasitic, is remarkably effective against a broad range of gastrointestinal and external parasites affecting humans while it has also been widely used in veterinary and aquaculture medicine to control Platyhelminthes. Specifically, in captive fish, PZQ has proven an effective chemotherapeutic against monogeneans infecting the gills, skin and branchial cavities. Orally administered PZQ preparations at doses ranging from 50 to 400 mg/kg fish daily for up to 20d have been tested in a variety of cultured fish species (Kim et al., 2001; Tubbs and Tingle, 2006; Shirakashi et al., 2012). In establishing an oral dosing regimen, pharmacokinetic data can be useful for optimising treatment efficiency. However, to our knowledge, there is no literature examining the pharmacokinetic properties of PZQ in gilthead seabream, *Sparus aurata* which is an economically important species in Mediterranean marine fish farming. Thus, the aim of this study was to investigate the pharmacokinetic properties of dietary administered PZQ in gilthead seabream following a single oral dosing as a first step to optimise PZQ dosing regimens for this species.

Materials and methods

Healthy gilthead seabream (52±3.7g), were obtained from a commercial fish farm and transferred to the experimental aquarium facilities. Fish were randomized in 2 groups of tanks; Low (n=100): fish were fed with an experimental diet supplemented with PZQ (75 mg/kg) and High (n=100): fish were fed with an experimental diet supplemented with PZQ (150 mg/kg). Before the experimentation, fish were acclimatized for two weeks and were fasted for 24h. During the experimental procedure, fish were hand-fed once a day, assuring that the feed was consumed. Water temperature was maintained at 21±°C. Ten fish/group were anesthetized (MS-222, 150ppm) and blood samples were collected each at 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96h post treatment. Plasma was separated from blood samples by centrifugation at 3000*g* for 10min at 4°C. A modified method of Ridtitid et al. (2002) was used for the extraction of PZQ in plasma samples which were then analysed by an HPLC-UV chromatographic apparatus.

Results

The concentrations of PZQ found in plasma after oral administration are shown in Figure 1. In detail, plasma PZQ concentrations in gilthead seabream revealed values around 6.3 μg/ml and 6.4μg/ml at 4 and 8 h post feeding, respectively for low dosing regimen and 7.9 and 8.3μg/ml at 1 and 4h post feeding, respectively for high dosing regimen. Maximum plasma concentration (C_{max}) of PZQ for fish fed 75mg/kg BW was achieved at 8h post feeding, while in fish fed 150mg/kg BW was evident at 4h post feeding (8.3μg/ml). These profiles indicate that the clearance of PZQ in gilthead seabream did not follow a simple decay model. Regarding drug clearance from fish circulation, PZQ concentration declined relatively sharply at the 12h sampling point (approximately 50%), however, C_{min} were found to be approximately 1μg/ml 96h post-treatment for both dosing regimens examined. Finally, a two-fold increase in the administered dose only led to an 46.5% increase in the mean plasma concentration of PZQ 2h post-treatment.

Discussion & conclusion

The results of the present study showed that PZQ was readily absorbed in plasma of gilthead seabream while its clearance was found to be relatively sharp for both examined dosing regimens. Rapid absorption of orally administered PZQ preparations has been also demonstrated in other fish species such *Seriola spp*. (Tubbs and Tingle, 2006, Kogiannou et al., 2021). Considering the sharp decrease in PZQ concentration in plasma after 12h after oral administration in the present study, re-treatment at an interval of 24h would possibly enhance eradication of *Sparicotyle chrysophrii*, a polyopisthocotylean gill parasite of gilthead seabream. In conclusion, based on the information obtained from the PZQ analysis in gilthead seabream plasma, there is an apparent benefit from the 150mg/kg dosing regimen, as confirmed by the significantly higher drug levels compared to low dosing. An *in situ* trial against *S. chrysophrii* is required to verify that the high dosing schedule suggested herein is also the most effective medicated scheme.

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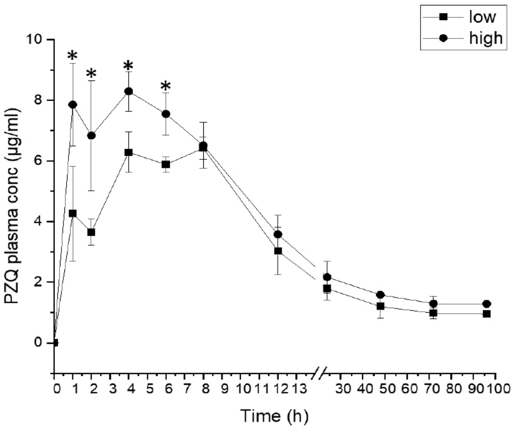

Figure 1. Plasma concentration (µg/ml) of two PZQ doses (low=75 mg/kg BW, high=150 mg/kg BW) in gilthead seabream following a single oral dosing. Values shown are mean±stdev (N=10). * indicates a statistically significant difference ($P < 0.05$).

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SEASONAL COMPARISON IN FILLET GAPING OF FARMED GILTHEAD SEABREAM (*Sparus aurata*) AND RED SEABREAM (*Pagrus magor*)

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Introduction

Gilthead seabream (*Sparus aurata*) is considered one of the most valued marine species produced in most Mediterranean countries, including Greece. It is mainly commercialized as whole fish, gutted or not. In recent years, the growing consumer demand for a high quality secondary processing product, i.e. fillet, has led to the beginning of production of sea bream fillets. This brought to surface technical quality issues, such as gaping of the fillets. Fillet gaps result to the downgrading of the food product, and hence economic loss, because of rejection by the consumers due to its unappealing appearance. Briefly, gaping is a phenomenon, in which the connective tissue fails to hold the fish fillet together resulting into gaps and tears at the myofibre–myocommata attachments and between myofibres. Factors that have proven to be strongly associated with the fish propensity to gap, include the species, the biological status, the catch or slaughter history, the temperature during storage (Lavety et al., 1988; Sheehan et al., 1996; Robb et al., 2000). Most studies in fillet gapping refer to salmonids. However, no respevtive data occur for sparidae species. Taking these into account, the aim of this original study was to investigate the seasonal variations of gaping in gilthead seabream fillets and its relative species red seabream (*Pagrus magor*), in order to gain insight into potential causes of gaping in farmed Mediterranean species fillets.

Materials and methods

Market-size (400-800g) gilthead seabream (total N= 274) and red seabream (total N= 186) were harvested from sea cage farms during three seasons (winter, spring and summer), slaughtered according to standard commercial procedures, packed with ice and shipped to a commercial fish processing facilities, where they were stored at 0-4^{o}C for up to two days. After mechanical scale removal by drum, fish were filleted, using a filleting-machine, weighted, ice-packed and transferred to the Hellenic Centre for Marine Research (HCMR). Fillets were assessed for their degree of gaping by a recently developed scale (Kogiannou et al., 2022), pH, water holding capacity (WHC), total composition and total collagen content. The SPSS version 26.0 was used for the statistical analysis. Comparisons among means were made using one-way analysis of variance (ANOVA). The non-parametric χ^2 test was applied to find statistical differences in the frequency of gaping occurrence between the seasons ($P<0.05$).

Results

Seasonal variations in gaping scores frequencies between winter, summer and spring for gilthead seabream and red seabream fillets are presented in Figure 1. For gilthead seabream, distribution of gaping scores frequencies were found to be statistically different among seasons. In particular, during winter and spring the same pattern was observed, while during summer period more gaping was evident (15% of fillets suffered severe gaping-score 5). On the contrary, for red seabream increased high gaping scores incidences were observed during winter as well as summer, while flesh integrity remained quite intact during spring.

Lower post-mortem pH values were found in both species during summer (Table 1). Regarding WHC (Table 1), significantly poorer liquid holding capacity was observed during summer than the other two seasons for gilthead seabream. Similarly, for red seabream also the higher WHC value was observed during spring. Furthermore, a positive correlation between WHC and pH values during the examined seasons was observed in both species. Finally, regarding the chemical parameters examined herein, seasonal variations in total composition and in collagen content for both species were observed.

Conclusions

The results of the present study showed that in Mediterranean species, fillet gaping occurrence is significantly affected by season and specifically a higher gaping problem was observed during summer for both species examined. Overall, the results indicate that there is a relationship between post-mortem fillet pH, WHC, fillet collagen content and fillet gaping, however, further investigations are required.

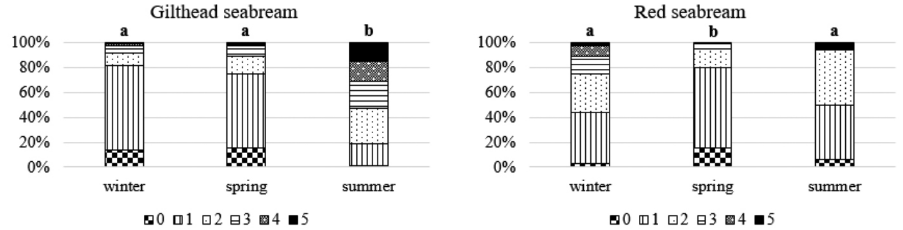

Figure 1. Distribution of gaping scores frequencies (%) of gilthead seabream (left) and red seabream (right) fillets according to season. Significance differences amongst season are indicated with different letters (*P* <0.05)

Table 1. Water holding capacity % (WHC) and pH values measured in gilthead seabream and red seabream fillets during different seasons (mean±st.dev). Significance differences amongst season are indicated with different letters (P <0.05)

species	parameter	winter	spring	summer
gilthead seabream	WHC	96,85±0,63^a	97,01±0,64^a	93,80±1,66^b
	pH	6,50±0,13^b	6,82±0,08^a	6,45±0,06^b
red seabream	WHC	96,87±0,85^b	98,34±0,22^a	94,92±1,43^b
	pH	6,70±0,08^a	6,46±0,06^b	6,31±0,11^b

Acknowledgement

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GENETIC DIVERSITY, EXTENT OF LINKAGE DISEQUILIBRIUM AND PERSISTENCE OF GAMETIC PHASE IN LUMPFISH *Cyclopterus lumpus*

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Introduction

Over the last few years, aquaculture industry contributes half of the global production of aquatic animals. Despite the great diversity in farmed aquatic species, Atlantic salmon (Salmon *salar*) dominates marine aquaculture production in volumes and production (Anrooy et al. 2022). Nowadays, the most pressing problem that salmon farming industry is facing is the infestation caused by the parasitic sea-lice (Lepeophtheirus *salmonis*) (Powell et al. 2018). As sea-lice have become resistant to chemical treatment, several species of cleaner fish are being used as the "biological solution" to the problem. Of them, the lumpfish (Cyclopterus *lumpus*) is looking as one of the most useful as it is easier to breed and keep feeding with see lice in low temperatures (Imsland et al. 2018). Moreover, is widely distributed in both sides of the North Atlantic Ocean and can survive in a wide range of temperatures. As a result, the demand for lumpfish has increased dramatically but most of the lumpfish used in salmon farming are still derived from wild broodstock (Winfield 2018, Powell et al. 2018). In this study, we analysed multiple wild populations, from the most common sources of lumpfish in aquaculture, and four commercial populations from a breeding programme in Scotland at Otter Ferry Seafish (OFS). The objective was to characterize multiple populations of lumpfish to analyse the impact of population structure and estimate the genetic diversity levels within and between the wild and the breeding populations.

Materials and Methods

A total of 631 animals, 500 obtained from the OFS and 131 animals from various wild populations (36 as breeding candidates), were used in this study. Four full sib lumpfish families from wild origin were produced in spring 2019 at OFS facilities and maintained in separate tanks. Fin clips were obtained from all the candidates and the four families including 125 random fish from each family. The ddRAD libraries were prepared, containing all the samples, and were sequenced on Illumina Novaseq 6000 platform. Reads were aligned against the genomic assembly of C. lumpus (NCBI Assembly accession GCA 009769545.1) using bwa v0.7.17 (Li and Durbin 2009) and assembled using Stack v2.41 (Rochette, Rivera-Colón and Catchen 2019)Rivera-Colón and Catchen 2019. Identified SNPs were retrieved from the samples and filtered for quality control, using PLINK software (Purcell et al. 2007), according to different criteria: such as: a) minor allele frequency$\geq$0.005, b) p-value of χ^2 test for Hardy-Weinberg equilibrium$\geq 10^{-6}$ and c) SNP and animal call rate $\geq$ 0.95. SNPs or individuals that failed to pass the criteria were removed. After the quality control filtering, individuals and SNPs that passed the criteria remained for further analysis. Genetic diversity metrics such as inbreeding coefficients and average pairwise genetic distance were used to estimate levels of within and between population genetic diversity. A principal component analysis was used to explore patterns of genome composition between populations and highlight the variation between them. Linkage disequilibrium (LD) levels and persistence of gametic phase disequilibrium (r) were used as criteria of investigation between the lumpfish populations. PLINK and GCTA were used to perform these analyses (Purcell et al. 2007, Yang et al. 2011).

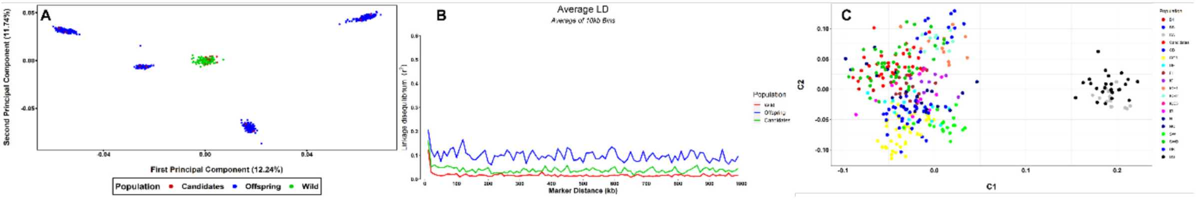

Figure 1 A) Principal component decomposition of the genomic relationship matrix coloured by population B) Average LD (r2) was calculated using 631 individuals within 10kb genomic bins, C) Principal component decomposition of the IBS coloured by location.

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Results

The comparison of the first two principal components clearly discriminates the four breeding families and their parents from the wild populations, but there is no evidence of differentiation across the wild populations. Similar levels of genetic diversity were observed among all the wild groups based on the average pairwise IBS distance (0.15 – 0.25). The breeding populations shows a high genetic differentiation between them (0.3) and higher across the wild groups (0.46) (Figure 1A). The estimation of the average LD (r^2) within 10 kb genomic bins and 1,000 KB of maximum distance between two SNPs, by using a subset of common SNPs shows that LD declined rapidly as the physical distance between marker pairs increases (Figure 1B). Both groups have LD values close to 0.2 in small distances but only the "Offspring" population retain extensive LD as the physical distance increases. The consistency of gametic phase was calculated by the Pearson correlation of r values, the higher values was observed between the "Candidates" and the "Offsping" group (Figure 1C). Moreover, the comparison of the genetic differentiation between all the wild individuals from 11 locations shows a distinct differentiation between the American and European individuals.

Conclusions

An overview of population structure, genetic diversity, LD decay and geographic differentiation multiple wild and breeding populations of lumpfish was obtained from the analysed data. Our SNP subset shows a good coverage of the genome and can discriminate the genetic difference between populations even by using a very small number of selected SNPs. Based the genomic information, the PCA shows a strong distinction between the breeding populations and the wild groups. The similar levels of genetic diversity that were observed among the groups indicate that there is a lack of genetic structure based on the origin in the European wild populations but there is a strong differentiation compared with individuals from the American continent. The lack of population differentiation between the groups suggests that there would be a small effect on the genetic diversity from lumpfish escapees. Moreover, the rate of LD decay and the low correlation of phase indicates that this panel will be efficient at estimating the genomic breeding values across the breeding populations.

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DIET AND REARING ENVIRONMENT EFFECTS ON MICROBIAL COMPOSITION IN THE GUT: THE CASE OF NILE TILAPIA

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Introduction

Fish harbour a high diversity of microbes in their gut, which plays a crucial role in nutrition and health through host-microbe interaction and microbe-microbe interactions (Wang et al., 2018). While microbes can profoundly affect their hosts, we are only beginning to understand how variation in the microbial colonisation through the rearing environment or the diet may shape host performance throughout life. Moreover, with the current trends in fish diet formulation and the substitution of fish meal with alternative ingredients, there is a higher carbohydrate inclusion, including non-starch polysaccharides (NSP), which are less digestible by fish (Kokou and Fountoulaki, 2018). Understanding how both the environment and the diet affect microbial composition and structure in the gut can assist in shaping rearing protocols, but also dietary formulations leading to better fish performance.

Microbial co-occurrence network analysis can be a powerful tool to explore the forces that affect microbial community structure and its dynamics. Such networks have been recently reported in fish to reveal antibiotic effects (Kokou et al., 2020) or understand microbial dynamics (Abdelhafiz et al., 2021). In this study, we explored the impact of early life rearing environment and diet on the microbial associations and composition in the gut of Nile tilapia (*Oreochromis niloticus*). Nile tilapia has omnivorous dietary habits and the potential for NSP fermentation (Maas et al., 2020), thus making it an interesting species to understand NSP effects on the gut microbiome.

Materials and Methods

Gut samples from two different feeding trials using Nile tilapia post-larvae and juveniles were analyzed for microbiome composition. In Trial I, post-larvae tilapia were exposed to different rearing environments (Biofloc, BFT vs Flow-through, FTS) during the first two weeks of feeding, then transferred to a common garden environment, and fed with two inclusion levels of dietary NSP (17%, M-NSP and 27%, H-NSP). In Trial II, fish were fed with high levels of dietary NSP (~31%, CON), supplemented with enzymes phytase and xylanase (ENZ), or probiotics (PRO), and the combination of the two (ENZ+PRO). For the microbiome analysis, DNA was extracted with commercial kits and the V4 and V3-V4 variable regions of the 16S rRNA gene were sequenced, respectively, using Illumina MiSeq. Taxonomic composition of the microbiome was performed at the single-nucleotide level. For diversity analysis, the '*microbiome*' R package was used. Co-occurrence network analysis (Kokou et al., 2020) was applied to explore microbial association patterns in the different treatments.

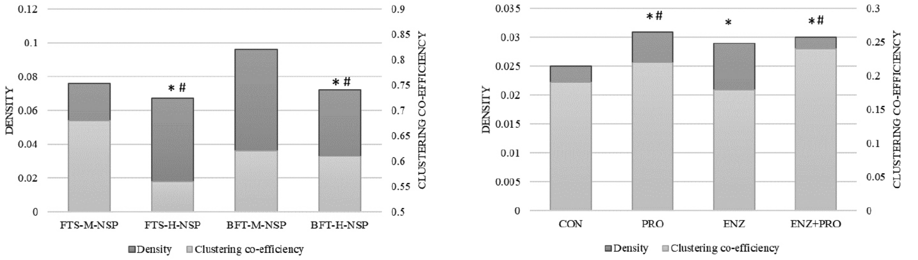

Figure 1. *The density and clustering co-efficiency of the co-occurrence networks in a. Trial I and b. Trial II. Stars and hash indicate significance for the density and clustering co-efficiency, respectively, between M-NSP and H-NSP in Trial I, and between control and the rest of the treatments (PRO, ENZ, ENZ+PRO) in Trial II.*

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Results

The results showed that diet and early life environment could affect the gut microbiome composition. Although the developmental stage had a substantial impact on the gut microbiome composition, we observed an effect of both the rearing environment (BFT vs FTS) and the diet on the gut microbial associations. In Trial I, early exposure to BFT led to a significantly higher ratio of positive to negative microbial associations. Moreover, we observed that H-NSP diets led to a significantly lower density of microbial associations (*number of connections between microbes*) and clustering co-efficiency (*the degree to which microbes cluster together*)(Figure 1a). In Trial II, the inclusion of enzymes or probiotics in high NSP diets led to a higher network density and clustering co-efficiency (Figure 1b). These results indicated that high indigestible NSP might interfere with microbial associations. The combination of enzymes and probiotics in the diets can have a complementary effect by increasing the availability of substrates for the probiotic and promoting the abundance of beneficial bacteria while increasing NSP degradation (Maas et al., 2020). Increased network complexity (clustering and density) with probiotics was hypothesized to benefit the gut microbiome homeostasis (Yang et al., 2017).

Conclusion

These findings suggest that diet and the rearing environment play a significant role in modulating the fish gut microbiome. At the same time, the latter is critical during early life stages. The level and potential availability of indigestible NSP in the diets consistently altered microbial networks. Although further exploration is needed to understand microbial networks to optimize their application in aquaculture, such analysis can serve as a tool to better understand microbial dynamics in the fish gut and how such dynamics may be altered by dietary formulations or rearing protocols, ensuring an optimal fish performance.

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EFFECT OF *Trypanoplasma borreli* INFECTION ON SPERM QUALITY AND REPRODUCTIVE SUCCESS OF COMMON CARP (*Cyprinus carpio* L.) MALES

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Introduction

A controlled fish propagation is nowadays the fundamental prerequisite for well-managed aquaculture production. However, despite practices aimed at creating equal conditions during mass spawning, in the condition of sperm overload and short sperm-egg distance the share of males giving offspring is highly diversified. In traditional pond aquaculture, the fish are exposed to various environmental factors that may affect their fertility through immune activation, among which parasites are one of the most influential. It is highly feasible that differences in fertilization success between males are related to sperm characteristics determined by fish fitness. The study aimed to investigate if mounting an immune response induced by parasitic infection affects common carp sperm quality and reproductive success (*Cyrpinus carpio* L).

Materials and methods

Mature carp males were experimentally infected with a dose of 1 x 10^9 blood parasites *Trypanoplasma borreli* by intraperitoneal inoculation. Control fish were PBS-injected. Parasite number was determined at weekly intervals using a Bürker counting chamber. The level of parasitaemia developed within four weeks post-infection was the criterion to classify each individual either as parasite susceptible or resistant. The measured level of *T. borreli*- specific antibodies production gave information on the fish's immune system efficiency. On day 24 post-infection, carp males were engaged in controlled spawning. Equal volumes of quantified semen, obtained by gentle abdominal massage, were used to fertilize the egg portions, obtained from four females of different genetic origins. The calculated hatchability rate was used as the indicator of the males' reproductive success. To evaluate the quality of fish sperm, we measured motility parameters using the CASA system and acridine orange staining for DNA integrity assessment. We also estimated the level of 11-ketotestosterone and its relationship with the antibodies production.

Results

The parasite-infected males demonstrated average hatchability rates of 14.18 ±4.83%, while the control males demonstrated 11.29 ±3.93% rates. Parasites induced a robust adaptive antibody response, but individual antibody levels were not associated with hatchability rate. A significantly higher number of spermatozoids with damaged DNA was detected in parasite-susceptible males than in the resistant ones. The 11-ketotestosterone and most CASA parameters did not differ between parasite-infected and control fish except beat cross frequency (BCF), which was higher in infected fish (57.45 ±18 Hz in infected vs 43.64 ±9 Hz in controls). This CASA parameter alongside the percentage of motile spermatozoa (MOT) was positively associated with hatchability rate.

Discussion

Our study shows that in artificial spawning, a mild level of parasitaemia does not negatively influence the reproductive success of common carp males. The BCF was the only sperm characteristic that "reacted" to the parasite infection, being the highest on average, among most susceptible males. At the same time, the parasite-susceptible group also presented the highest percentage of damaged sperm cells. We suggest that in the conditions of high egg/sperm ratio practised in aquaculture, the negative effect of sperm with damaged DNA can be diminished. In this way, the maximum fertilization potential was exposed. The association of BCF values with carp susceptibility to *T. borreli* infection and with the hatchability rate were the most important relationships among our results. It seems that susceptibility to parasitaemia affects some sperm characteristics, which are manifested through the higher BCF values. However, the mechanism by which the activated immune system can alter BCF values and why this sperm parameter affects hatchability rate remains unknown and requires further investigation.

AUTOMATED FISH MONITORING AND SAMPLING IN RAS USING OAK-D

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Introduction

The monitoring of fish for stock assessment in aquaculture and commercial fisheries is essential for the economic and environmental management. Measurement of fish mass as one of the important stock assessment features, is one of the most common and important practice in the aquaculture management. Fish mass information is playing important role in feeding regimes management, oxygen consumption calculation, antibiotic dose, grading time and the optimum time of harvest. Moreover, fish mass measurement has become more critical in recirculating aquaculture system (RAS) which recently became popular among fish farmers (Bergheim et al., 2009). The most conventional method to estimate the mass of a fish population is to net fish samples from a tank and weight them. This method is labour-intensive and stressful for fish. The possibility to measure the fish in a tank without human intervention is therefore of great interest for the aquaculture community. Machine vision system as non-invasive technique is attracting researcher and agriculturists for estimating fish mass and size during cultivation remotely without causing stress in past three decade (Saberioon et al., 2016; Zion, 2012). Usually, it is complicated to transfer the images from the tank camera to the central computer at the aquaculture facility and it became more problematic with the high number of tanks. The need of individual camera for each tank and processing of all data on the central computer makes the system difficult to apply under the real aquaculture conditions. The solution could be to detect the fish, estimate the weight and send the information to the central computer (wireless connection can be used). OpenCV AI Kit with Depth (OAK-D) can effectively solve the described problems. The individual fish is detected using the CNN. The stereo vison system is used to determine the fish depth and estimate weight. The main objective of this study was developing real time monitoring system using OAK-D for remote and automatic estimation of fish biomass in RAS.

Materials and methods

The study was divided into a several phases:

Data collection
OAK-D was mounted on top of fish tank for initial data collection. At first OAK-D was calibrated with a checkerboard (9*6 internal corners and square size is 2.35 cm) according to DepthAI Docs. Disparity map was not good, especially at distances of 2,5 m. In this range of depth, we are using OAK-D in our system. So, calibration procedure was redone with the checkerboard (8*6 internal corners and square size is 9 cm), because DocsAI protocols couldn't detect internal corners of smaller checkerboard at that distance.

Lights condition is very crucial in which our system work. As the water should be transparent enough for good fish detection light conditions source were adjusted. The common carp is using as a testing specie. For estimation biomass and individual fish weight and length/width conversion, we sampled all fishes (Table 1). Each individual fish was captured by OAK-D at closest depth to the surface. Several videos were recorded with different light sources. These data were manually processed and labeled in LabelImg v.1.8.2. Two training data set were created for individual fish detection with 800 of photos each with different resolutions. Tiny Yolo V3 Fish Detector was trained with 416*416 resolution. Second detector was based on SSD MobileNet V2 and it was trained with resolution of 300*300.

The new data set of 2000 photos (300*300 resolution) with different light conditions was sampled. All photos were annotated in a simple graphical tool – imaglab which is part of Dlib library. The data were randomly split into training and testing sets in proportion 90 and 10% respectively. The SSD MobileNet fish detector was trained.

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To localize individual landmarks on detected fish we used well-known dlib's shape predictor. dlib's find_min_global function was used to optimize the options and hyperparameters to dlib's shape predictor training. Dlib handles computing the mean average error (MAE) between the predicted landmark coordinates and the ground-truth landmark coordinates.

For conversion pixel size to real length system was calibrated with special white plate. It was captured 10 photos at 3 locations in tank: middle, edge and between by step 10 cm lowering this plate. The distance to the plate was measured with 5 m ruler. The conversion equation was calculated by fitting a linear model.

Implementation and testing performance
SSD MobileNet model was compiled for 4, 5, 6 shaves. After both detectors were applied we had detection of reflections without fish and wrong detections of landmarks. To avoid misestimations of size we used several conditional statements which were calculated according to our data set. These conditions are distance to bounding box and distance between landmarks, as we know real size of our fish. Weight of each detected fish is calculated by conversion models. Number of fish (N) to the day of recording video in the tank was 21. Total biomass was calculated by multiplying average weight of fishes on N.

Stereovision provided distance to the detected fish.

Results and discussion

This automated monitoring and sampling system can help farmers monitor fish welfare and growth rates in high intensity cultivation systems such as RAS continuously in real-time. The SSD MobileNet fish detector showed better performance than Tiny Yolo. The lost on last step of the SSD MobileNet training was 1.32. Best performance was gotten on 6 shaves blob file. FPS is around 16. Dlib MAE of training and testing set was 3.5 and 15 respectively. Despite not so well depth map under water OAK-D shows sufficient performance. As far as we know that it is no such system at the market. It can increase cultural efficiency and productivity. More statistic data will be acquired, and that result will be published in corresponding scientific journal.

Acknowledgement

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GOOD AQUACULTURE PRACTICES; THE COLLABORATIVE USE AND BENEFITS

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The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) is a collaboration between the United States Food and Drug Administration (FDA) and the University of Maryland (UM). JIFSAN has developed an aquaculture program that links food safety and disease prevention. It's called Good Aquaculture Practices (GAqPs). GAqPs are widely used and implemented around the world. This presentation is a short synopsis on GAqPs and a request for to help take GAqPs to the next level where it can be universally used and recognized for a wide variety of purposes. GAqPs could be used or integrated into new or existing programs to show food safety and disease controls are in place by the following:

- Individual aquaculture farms for their buyers;
- Governments for their farm and or processor registration and certification programs;
- Private third-party certification programs to verify food safety and disease prevention;
- Processors in their HACCP or Preventative Control programs to act as their food safety controls for aquaculture related food safety hazards;
- Academia to train future aquaculture professionals in food safety and disease prevention;
- The aquaculture industry and others to demonstrate to the general public that aquaculture products are safe, sustainable, and free of hazardous residues and pathogens.

NERVE REGENERATION IN CRAYFISH

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Some species ranging from protozoans to mammals can regenerate their organs after an amputation or injury. Crayfish, as the representative of Decapoda, the most diverse order of crustaceans, exhibits substantial abilities for regeneration. Especially the clonal marbled crayfish (*Procambarus virginalis*) poses an excellent model for understanding arthropod regeneration. Antennae in crayfish are essential for gaining tactile information for sensing the local topography, localizing food and communicating with conspecifics. Therefore, the antennae are sensory organs full of nerve networks, making them a good candidate for the study of nerve regeneration. Crayfish haemolymph contains immune cells which can turn into stem cells and participate in the nerve regeneration in the brain of crayfish. Therefore, we focused on the potential roles of immune cells in the regeneration of antennae at the ultrastructural level using transmission electron microscopy. Our observations showed that immune cells could turn into nerves and provide essential organelles such as mitochondria and Golgi apparatus, which are necessary for the functioning of neurones. In conclusion, we reported morphological changes in immune cells as stem cells during nerve regeneration in crayfish.

PREDICTING THE EVOLUTIONARY TRAJECTORIES OF PARALOGS OF THE COMPLEX V OF OXIDATIVE PHOSPHORYLATION IN *Dicentrarchus labrax*

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Introduction

European seabass (*Dicentrarchus labrax*) is a teleost of great economic and cultural importance in the European Union and the Mediterranean, since it is the second non salmonid cultivated species in production volume in the European Union. Teleosts have undergone three rounds of whole genome duplication (WGD). It is believed that the first round of WGD happened early in the Vertebrate lineage (1R), the second after the differentiation of Agnatha and Gnathostoma (2R) and the third (3R) is the Teleost Specific Whole Genome Duplication (TS-WGD) (Kasahara, 2007). After a WGD event, decreased evolutionary pressure allows the paralogs that have been formed to follow different evolutionary trajectories, such as non-functionalization, sub-functionalization or neo-functionalization. Oxidative phosphorylation is a metabolic process, responsible for the 90% of cellular energy production through the synthesis of ATP. Five complexes in the inner mitochondrial membrane take part in this process, with the first four forming the electron transfer chain. The proton gradient generated by the electron transfer chain is used by the complex V or ATP synthase to produce ATP. It is clear that the efficiency of energy production through oxidative phosphorylation is crucial for the fish wellbeing, especially for energy demanding processes such as development and growth. The aim of this study was to explore how the TS-WGD has affected the process of oxidative phosphorylation with focus on the ATP Synthase.

Materials and Methods

The first step in the analysis was database mining for the genes atp5mg, atp5pd, atp5pf and atp5if1 that encode the ATP synthase subunits g, d, F6 and the inhibitory factor IF1, respectively. Using the databases Ensembl and NCBI, sequences of paralogs for the genes of interest were retrieved and their homology was verified using Ensembl's BLAST algorithm. The sequences were first edited and then used in the phylogenetic analysis where phylogenetic trees were created for each gene family, using the Maximum Likelihood Method. The species used were Teleost representatives, Sarcopterygii, Tetrapods representatives, the species Elephant shark and Lamprey, which were used as root for the trees, and Spotted gar, whose lineage differentiated before the TS-WGD. For synteny analysis, circos diagrams (Krzywinski et al., 2009) were created for each gene family, where 50 genes on either side of every gene of interest were mapped on chromosomes, to visualize the level of conservation between orthologous genes on chromosomes of the species European seabass, Spotted gar and Human. The online algorithm Pseudochecker (Alves et al., 2020) was used for pseudogene prediction for European seabass paralogs, using stickleback sequences as reference. TargetP (Armenteros et al., 2019) was used for mitochondrial transit peptide and MPP cleavage site prediction. Finally, the protein database Uniprot was used to identify domains related to the protein structure and function on reviewed human proteins. The human sequences and domains were then compared to those of the European seabass.

Results and Discussion

Different paralogs were identified for every gene and were named based on the phylogenetic analysis. Three paralogs were identified (α1, α2, β) for *atp5mg*, two (α, β) for *atp5pd*, three (α, β, γ) for *atp5pf,* and another three (α, β1, β2) for *atp5if1*. Based on the phylogenetic and synteny analysis the evolutionary origins of the paralogs of each gene family were identified. The paralogs of *atp5mg* and *atp5pd* seem to have originated from TS-WGD. The α paralogue of *atp5pf* originated from 2R duplication, while β and γ, from TS-WGD. The paralogs of *atp5if1*, originated from 2R duplication. Lineage-specific gene duplication seems to be the origin of *atp5mg* α1 and α2, and *atp5if1* β1 and β2. The synteny analysis indicated conservation between the three species chromosomes that contain the paralogs of each gene family, except for the chromosome that contains the *atp5mg* β paralogue and the one that contains the *atp5pf* γ. The prediction for pseudogenes, for mitochondrial transit peptide as well as structural and functional peptide domains were taken into consideration for the prediction of the evolutionary fate of each paralog. The peptide sequences of the genes *atp5mg* α2, *atp5pf* β, *atp5pf* γ and *atp5if1* β2, were predicted to be so differentiated in comparison to the reference sequences, that they cannot accomplish the same function. An absence of mitochondrial transit peptide was observed in the *atp5mg* α2, *atp5pf* γ and *atp5if1* β2.

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The parts of *atp5pf* γ and *atp5if1* β2 that in the human peptides contain the mitochondrial transit peptides, seem to have been lost. The β2 paralogue of *atp5if1*, seems to have also lost its functional domain, while in *atp5pf* β, an extra region of about 300 amino acids after the mitochondrial transit peptide that does not appear in the human corresponding peptide, makes the protein three times longer. The results, show that there are different possible evolutionary trajectories between the paralogs of each gene family. Non-functionalization seems to be the case for *atp5mg* α2, *atp5pf* γ and *atp5if1* γ. Regulatory sub-functionalization appears to be the fate of *atp5mg* α1 and β, *atp5pd* α and β and *atp5if1* α and β, as they seem to maintain their functions. For the β paralogue of *atp5pf*, there seems to be an indication for neo-functionalization as it appears to maintain function, yet a large sequence region has been inserted after the mitochondrial transit peptide. Lastly, the only paralog that seems to have retained its ancestral function is the α of *atp5pf*. In the future, experimental evidence is necessary for validating the results of *in silico* analysis for the genes of ATP synthase and also other OXPHOS complexes to discover the influence of TS-WGD in oxidative phosphorylation.

Acknowledgements

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MITOCHONDRIAL SEQUENCE DIVERSITY IN THE *Carassius auratus* COMPLEX IN HUNGARY

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Introduction

Invasive gibel carp, *Carassius gibelio* (Bloch, 1782) has become well established in the Hungarian waters and now are spreading in European waters. It belongs to the Cyprinidae family, the biggest freshwater fish family, and the genus *Carassius* is native in East Asia. The circumstances of its initial introduction to Europe are still unclarified. The species' mass invasion dates back to the second half of the 20th century, which was facilitated by human activities such as intentional introduction for creating aquaculture and accidental introductions with common carp stock and their high ecological tolerance and ability of gynogenetic spawning. On major concern now is the potential hybridization between gibel carp and the other invasive species in the *Carassius auratus* complex (CAC), which may further accelerate the spread of the whole invasive species complex. The identification of gibel carp and their hybrids is difficult because of their morphological similarity to the other species in CAC. Here we carry out a genomic assessment to understand the history of gibel carp invasion and its phylogenetic relationship with the other species in CAC.

Materials and methods

One hundred and thirty-two gibel carp samples were collected from six locations in Hungary including Lake Balaton, two reservoirs of Kis-Balaton Water Protection System and Hőgyész, Siófok-Töreki fish pond system and Öszödi-berek. Following phenotypic identification, three loci of the mitochondrial genome (D-loop, Cytochrome c oxidase I (CoI) and Cytochrome b (Cytb)) were used to determine the phylogenetic origin of individuals and relationships among six gibel carp populations and the other species in the CAC. Successfully identified haplotypes were aligned with reference sequences in nucleotide databases (i.e., NCBI-BLAST: National Centre for Biotechnology Information and BOLD: Barcode of Life Data System). The phylogenetic relationships among gibel carp populations were then analyzed together with the reference sequences to understand the relationship and the level of hybridization with the species in CAC. Haplotypes diversity was calculated based on the polymorphic sites and haplotypes by using the DnaSP version 5 (Librado & Rozas, 2009) software. Pairwise FST, genetic distance and a phylogenetic tree was reconstructed with the Neighbour Joining method by using the MegaX software (Kumar et al., 2018). The network of the D-loop haplotypes was built using a median-joining algorithm byPopART software (Rohl, Bandelt & Forster, 1999)

Results

Among the 132 aligned D-loop sequences 22 haplotypes were identified based on 43 polymorphic sites. For each population, the highest haplotype diversity was in Siófok 0,83 (HD) ± 0,04 (SD), the lowest value was 0,19 (HD) ± 0,11 (SD) for the population of Öszödi-berek. The identified haplotypes were divided into three major groups on the haplotype network figure. The largest group contained sixteen haplotypes (all the common haplotypes belonged to this group), the second contained five low-frequency haplotypes, while the third group contained only one haplotype from our sequences. This latest clustered together with Carassius auratus buergeri reference sequence, then the other Carassius gibelio haplotypes did.

Further examination of representative individuals of the 22 haplotypes, only six Cytb (1057 bp region with polymorphic sites 27) and four CoI (562 bp region with polymorphic sites 26) haplotypes were detected. The largest number of haplotypes of all three loci were found in Lake Balaton, the largest shallow lake in Central Europe. Based on the NCBI-BLAST alignment of the D-loop, haplotypes of *Carassius auratus auratus* and *Carassius a. buergeri* in CAC were identified in the analys*ed* samples. The analysis of Cytb and CoI haplotypes confirmed the occurrence of intragenus hybridization of *C. gibelio* in the Hungarian waters.

(Continued on next page)

Discussion and conclusion

This study is the first genetic diversity assessment for the Hungarian gibel carp complex, in which we reported the recent homogenous genetic background of the studied populations. However, the potential hybrid origins of gibel carp were identified in the studied waters. The origin of the introgressed *C. auratus* sequences is doubtful but denotes the unreliability of morphological-based identification of taxa within the genus *Carassius* and the hidden presence of goldfish in natural waters of Hungary.

The Cytb and CoI have the lowest mutation rate among the mitochondrial protein coding genes in fish. The CoI is used in BOLD system for species identification but our results revealed that Cytb can be used more efficiently for identification the CAC complex because of more reliable databases. This marker showed the highest agreement with the phenotypes. While the D-loop showed the highest genetic variability. It is in agreement with other studies and explained by the highest mutation rate of the only larger non-coding mitochondrial region. It is much more informative than the nuclear markers when used for the analysis of closely related species, subspecies categories or populations. Our results also show that this region can be efficiently used for intrapopulation analyses for identifying hybrids in CAC.

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THE ATLANTIC SALMON IMMUNE SYSTEM CHANGES DURING SMOLTIFICATION: ENHANCED ACTIVITY OF B CELLS

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Introduction

Smoltification of farmed Atlantic salmon (*Salmo salar*) is associated with down-regulation of multiple immune genes, as has been shown in several experiments and field studies (1-3). Consequences of these changes remain unknown, although a higher activity of the immune system is not always associated with better resistance to pathogens (4). However, immune suppression can be partly responsible for high mortality of salmon during first months in the sea. In contrast to a general trend to decrease, we noticed enhanced expression of *immunoglobulins* (*ig)* and markers of B cells in smolts. Sequencing of the variable region of *igm* (Ig-seq (5)) has provided novel insights into the repertoire of antibodies and B cells and discovered previously unknown features of the teleost immune system. In particular, we have shown that responses to pathogens and vaccines are associated with increased B cell traffic, which enhances efficiency by delivery of antibodies to the sites exposed to the antigens (6). Increased mobility of B cells in Atlantic salmon smolts was shown in the field (7), but it remained unknown whether the observed changes were associated with smoltification or vaccination. Here, we report results of a controlled trial.

Materials and methods

A total of 330 unvacinated Atlantic salmon parr (~ 20 g) were randomly distributed among three freshwater recirculanting aquaculture systems (RAS) described in (8). The parr were smoltified over 14 weeks using a standard square wave photoperiodic regime. Subsequently, fish (~100 g) were transferred to a seawater flow-through system and grown for an aditional 12 weeks at LD 24:0, until ~500 g. Head kidney, spleen, visceral and dorsal fat, and gill tissues were collected at week 0, 8, 14 and 26.

Ig-seq was performed as described in (6). In brief, cDNA synthesis primed with an oligonucleotide to the end of *igm* constant region was followed with two PCRs that amplified the variable region (CDR3) and inserted adaptors for Illumina sequencing (MiSeq) Each unique sequence of the variable region or clonotype serves as a barcode of clonal B cells and the numbers of reads reflect the size of clones. Co-occurrence of clonotypes in two tissues is explained with traffic of B cells. Ig-seq was performed in the lymphatic (head kidney, spleen) and peripheral (visceral and dorsal fat, gill) tissues. *Membrane* and *secretory igm* expressed in respectively naïve and antibody producing B cells, *igt*, *igd* and gene-markers of immune cells were analysed with RT qPCR.

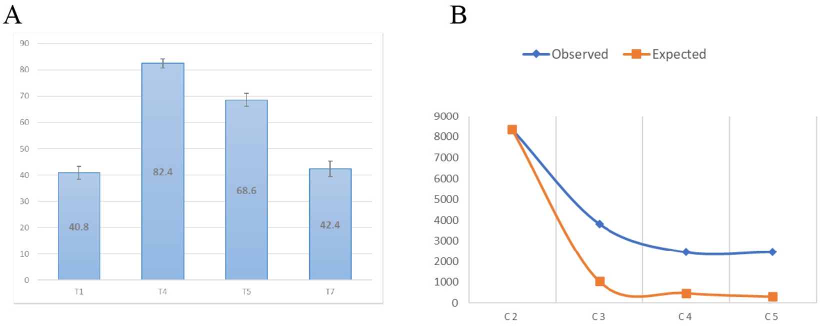

Figure 1. Co-occurrence of *igm* clonotypes reflecting traffic of B cells between organs and tissues of Atlantic salmon. **A**: cumulative frequency (%) of transcripts from clonotypes detected in more than one tissue. **B**: expected (random) and observed co-occurrence of clonotypes.

(Continued on next page)

Results and Discussion

Initial co-occurrence of clonotypes in tissues was relatively low, which is common for Atlantic salmon under basal conditions – the repertoire of B cells is unique at each site (Figure 1A). At T4 (light stimulation) sharing increased two-fold and 82.4% of all *igm* transcripts were from B cells clones detected in more than one tissue. This metric decreased at T5 (prior seawater transfer) and returned to initial levels at the last time-point (T7, 12 weeks after seawater transfer). High co-occurrence of clonotypes in several tissues (Figure 1B) suggested the simultaneous distribution of newly emerged B cells from the lymphatic tissues throughout the body. Active traffic was not associated with changes in the differentiation status of B cells: expression of *membrane* and *secretory igm*, and genes – regulators was not different between the time points. The qPCR confirmed high immune activity of visceral fat with expression of all genes higher than in gill, except for cd28, which is a T-cell marker.

Conclusion

In addition to the dramatic changes in the endocrine system, osmoregulation, metabolism, morphology, and behaviour, smoltification causes remastering of B cells population followed with its subsequent homogenization in the lymphatic and peripheral tissues. Repeated observations in the field and experiments suggest that this is an integral part of the developmental program. The driving force behind this restructuring, its functional importance and implications for aquaculture are waiting for exploration.

Acknowledgements

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VISUALIZING THE SOCIAL IN AQUACULTURE ACROSS GEOGRAPHIC SCALES

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Introduction

The United Nations Sustainable Development Goals (SDGs) can be viewed as a bold commitment to produce a set of universal goals that meet the urgent environmental, political and economic challenges of our time, among which food security is at the fore (UN, 2015). To this end, governments of many countries, as well as their supporting organizations, have primarily addressed the biological, technical and economic aspects of aquaculture as one central pillar for food security. In contrast, social and cultural aspects of aquaculture production have taken a backseat. Drawing on the observation that aquaculture development in Western Societies has largely failed to address these social effects across different scales and contexts, this presentation offers a new way of capturing and visualising the diverse social dimensions of aquaculture.

Material and Methods

The UN social dimension categories (Population (P), Health (H), Education (E), Work (W), Housing (HO)) were selected as a point-of-departure, under which each of these umbrella categories a number of sub-categories were defined, i.e. social equity, working conditions, ownership of housing, access to food, etc. Local/regional stakeholder knowledge realms were then combined with scientific expert knowledge to assess aquaculture operations across the North-Atlantic realm (ICES region) against these indicators.

In our case study examples, we focussed on two types of aquaculture production systems, rope mussel (Blue mussels, *Mytilus edulis*) and net-pen finfish (Atlantic salmon, *Salmo salar*), as these were present in all of the case studies selected and represented typical aquaculture set-ups within the respective country for these types of production systems.

Results

The approach indicates that one needs to have a minimum farm size in order to have an impact of a visible scale for the different social dimension categories. The relevance of the finfish farming industry to population is higher on a regional than a local scale in all cases due to job opportunities and associated increased social and economic activity. Despite that finfish aquaculture thus seems to be more social impactful than rope mussel farming, the latter can hold important cultural values and contribute to place-based understanding, connecting people with place and identity, thus playing a vital role in maintaining the working waterfront identity. lt could be shown that aquaculture boosts a potential significant pull-factor to incentivise people to remain in the area, keeping coastal communities viable.

The simultaneous summative visualization of the five social dimension indicators provides a holistic analysis of the impact of both forms of aquaculture production at the local, regional and national scales. It can be shown that there are multiple relationships, effects and trade-offs that warrant consideration if sustainable aquaculture in relation to the United Nations SDGs is taking place.

Conclusions

By visualising the social effects of aquaculture, a door may be opened for new narratives on the sustainability of aquaculture that render social license and social acceptability more positive.

SPATIAL SPILLOVERS IN PRODUCTIVITY DIFFERENCES OF SALMON FARMS

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Introduction

The main existing regulation in the Norwegian salmon aquaculture industry is a limitation on production at both farm and regional level (maximum allowable biomass and traffic light system). The rationale behind these regulations is to control the level of sea lice and avoid the prevalence of the disease. However, it is a broad measure, and limited research has examined such factors as the farm's regional location and mutual location of farms within a region due to the lack of geodata availability. The purpose of this study is to explore the causes of differences in farms' productivity while accounting for missing spatial determinants. The methodology is twofold. The first step includes the estimation of the farms' productivity following the Levisohn and Pertin semi-parametric approach. The second stage analyses the spatial panel data for the existence of spatial spillovers to the efficiency of other neighbouring farms.

Research significance

According to the existing regulations, limitation of production should put under control the level of sea lice. Nevertheless, this link is not trivial, as shown in Figure 1. There is no clear evidence that low production levels are associated with a low level of sea lice. Such factors as sea temperature might play a key role in farm efficiency. They are defined by farm location.

The investigation in this direction gives a better understanding of what kind of regulations the salmon industry is needed.

Methodology

Productivity of a farm is a good reflector of the farm decision-making process regarding both outcomes: a more efficient farm means that it produces enough to maintain in the market despite (or thank to) the regulations against a bad outcome – externalities. Therefore, the first stage of analysis is productivity measurement of the Norwegian salmon aquaculture industry using Total Factor Productivity (TFP) analysis. It can be done using a semi-parametric approach following Levinsohn, et al., (2003) and Ilke Van Beveren, (2010). Having a distribution of the farm productivity, the next step is to study spatial differences in TFP among regions that might be taken place due to different measures against externalities, and regulation application using spatial econometric analysis (Anselin, 2001).

The first step of the production estimation is done by recovering Solow residual from the natural log of the classic Cobb-Douglas function:

$$\hat{\omega}_{it}=y_{it}-\hat{\beta}_k k_{it}-\hat{\beta}_l l_{it}-\hat{\beta}_m m_{it}$$

Where ω_{it} represents firm-level productivity, y_{it} is the output of firm i at time t, and variables k, l, and m are inputs of capital, labor, and materials (feed costs, operational costs, etc.), respectively.

The second step estimates the spatial Durbin model to examine the influence of spatial spillovers and firms' treatment efforts on total factor productivity. The generalized form of the spatial dynamic models is as follows:

$$y=\varrho Wy+X\beta+\theta WX+\mu_i+\varepsilon_{it}$$

Where y is TFP, ϱ denotes spatial autocorrelation coefficient, W is a non-negative spatial weight matrix, X represents the independent variables, β and θ are the spatial regression coefficients. Wy,WX denote the spatial lag terms of the dependent variable and independent variables, which allow us to analyse the spillover effects of independent variables.

Preliminary results

Figure 2 illustrates preliminary results of the estimated productivity of salmon aquaculture farms from 2012 till 2018. As we can see there is still room for less efficient farms to evolve to the level of most productive farms.

The second part of the research would be devoted to the determination of whether there are spatial spillovers among the factors driving productivity differences.

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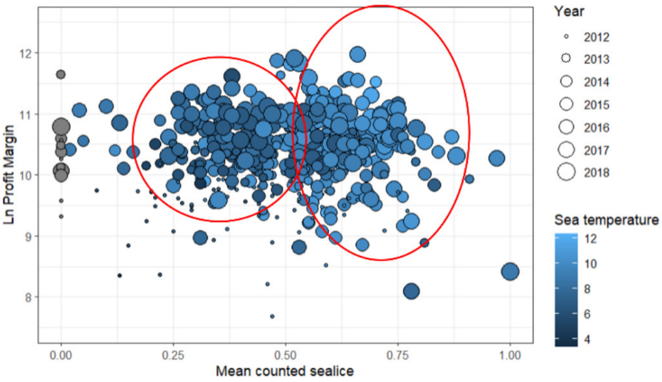
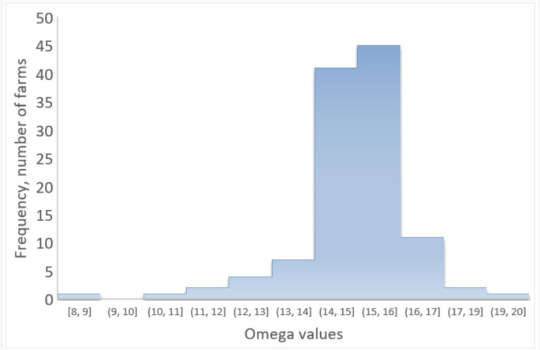


Figure 1 Link between a number of sea lice and farms' profit

Figure 2 Estimated productivity distribution



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HYDRAULIC IMPACT ON FISH MIGRATION IN SARIAKANDHI FISH PASS OF BANGLADESH

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The importance of open water fish in our socio-economic regime has recently drawn the attention of the policy makers of the country. FCD/FCDI projects mainly serve the agricultural interests, but it interfere fish migration. This inevitably affects the open water fisheries sector as migratory routes. Nursing grounds of many species of fish are hampered and disturbed for these projects also. In order to permit fish migration in rivers, it is necessary to maintain conditions that help migrants reach their spawning grounds. To overcome obstacles, such as hydraulic structures, placed in the path of migrating fish, structures must be designed to assist the fish to pass them. The periodic and directed travel of fish mainly for feeding, breeding and over coming adverse climatic conditions is called migration. Fish passes are constructed to allow normal breeding migration and to ensure natural route of fish movement.

The concept of a fish passes is relatively new in Bangladesh. At present, two Fish passes and two fish friendly structures are constructed. These are Fish Pass in Jamuna to Bangali River at Sariakandi in Bogra, fish Pass in Kawadighi Haor of Monu river in Moulovibazar, fish friendly structure in Lohajong river of Tangail and fish friendly structure at Morichardanra in Chapainawabganj. Fish fry, spawning and hatchling movement from Jamuna to Bangali River was the main objective of Sariakandi Fish Pass Project. The Fish Pass Project of Sariakandi is necessary for the development of the dominant fishes like catfish and small fishes. The structures will also aid in efficient development of the carp fishes. Spawning migration, mainly in carp fish, in the study area was found to begin at the 2nd week of May and continue up to the 3rd week of July. Catfish migrations began at the last week of March and continue up to the 2nd week of June.

Fish fry and hatching movement from Jamuna to Bangali river was the main objective of Sariakandi fish pass project. The study also found that there were seven major category migratory species in the project area and the fish pass is contributing positively for growth of fishery resources in then study area. During the monsoon carp fish is the dominating migratory species. Carpfish migrates in a higher velocity, whereas, catfish migrates in a lower velocity. Some problems were found in the operation and management of fish pass.

ACROSS SPAWNING SEASON VARIATION OF SPERM QUALITY IN FARMED ARCTIC CHARR (*Salvelinus alpinus*)

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Introduction

Arctic charr (*Salvelinus alpinus*) is a species of high economic value for the Nordic aquaculture. The highly variable reproductive performance that is commonly observed in commercial farms is hindering the industry expansion in Sweden. Semen quality characteristics such as motility-related traits and sperm concentration can influence male fertility in farmed fish species. Change in sperm quality across the spawning period is common in fish and can affect their reproductive success (Bobe & Labbé, 2010; Mylonas et al., 2003)in order to monitor changes in sperm quality parameters during a whole spawning period. On11January 2001,60% of the fish were spermiating, increasing to100% in mid-February and dropping to 30% by mid-April. Sperm density showed a slight increasing trend, with mean values ranging between 8.6 and 23.7 Â10 9 spermato-zoa mL À 1. Sperm motility percentage exhibited a sig-nificant improvement during the spawning season (analysis of variance (ANOVA. The present study aimed to investigate variation in sperm quality in Arctic charr breeding candidates across the whole spawning season.

Materials and methods

Arctic charr males from the Swedish breeding program (2017-year class) were used in the present study. Semen sampling was performed three times across the natural spawning season (n = 71; early October – November). Additionally, three samplings were performed in males kept under photoperiod conditions resulting in late spawning (n = 77; late October – December). Evaluation of progressive sperm motility (PM, %) and sperm kinematics, including average path velocity (VAP, µm/s), curvilinear velocity (VCL, µm/s), and straight-line velocity (VSL, µm/s), was performed by using a computer-assisted sperm analysis (CASA, Microptic) system and the SCA®Motility imaging software. Furthermore, sperm concentration (SC, $\times 10^9$cells/mL) was measured using a NucleoCounter® SP-100™ (Chemometec). Descriptive statistics were computed for the CASA-system parameters using statistical R (v.4.0.2). Across-season sperm quality variations were analyzed by non-parametric tests for comparing either two (Wilcoxon test) or multiple groups (Kruskal-Wallis test). Finally, Pearson correlation was used to determine relationships between sperm quality parameters across the spawning season.

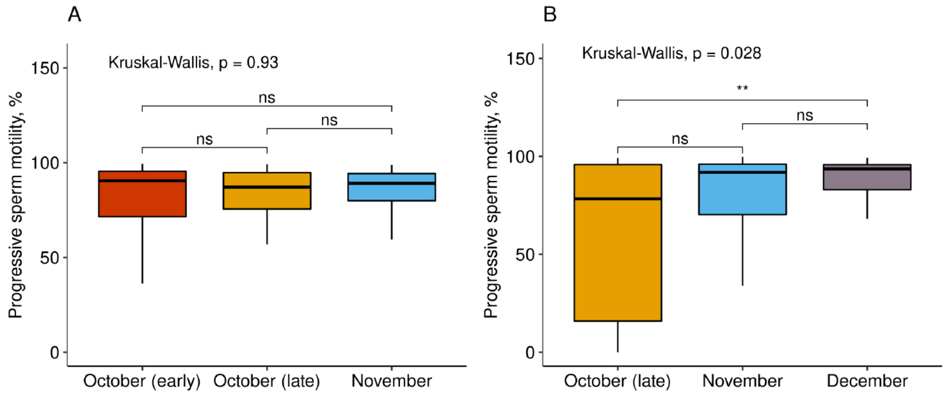

Fig. 1 Progressive sperm motility of Arctic charr males at different times across the spawning season in the groups with the natural (A) and delayed (B) spawning. Wilcoxon test, *P<0.05, **P<0.01, ***P<0.001, ns indicates non-significant difference in means.

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Results

Overall, there was a positive trend regarding the mean sperm quality traits in both natural and delayed spawning groups. More specifically, sperm concentration increased by 38% from late October to November in the natural spawning group. In the delayed group, sperm density increased by 39% from October to November and 133% from October to December. Sperm motility parameters increased by 4-13% in October and 8-17% from October to November in the natural group. In the delayed group, the increase was 14-28% from October to November and 38-45% from October to December (Figure 1). In addition, about 30% of the studied males exhibited minor individual variability in sperm quality with the standard deviation for SC $< 0.6\times10^9$ cells/mL and for PM < 5% during the spawning season.

Moderate to high correlations (0.31-0.68, P<0.01) were estimated for sperm concentration measured at different times across the spawning season. While for motility-related parameters, the Person correlations were between 0.41-0.54 (P<0.001) for recordings measured in late October and November in the natural spawning group and between recordings taken in November and December in the delayed spawning group. In the case of the remaining time points, the recorded motility parameters showed low correlations (–0.01-0.26, $P \geq 0.05$).

Discussion and Conclusion

In the present study, the across-season variation in the recorded sperm quality parameters was evident, showing, in general, a positive trend across the sampling points. These variations can explain to a certain degree the highly variable reproductive performance of Arctic charr in captivity. In addition to that, the individual variability in semen parameters during the spawning period was observed among the studied males. Although the reason for that remains poorly understood, environmental factors and genetic background are considered as crucial contributors to male reproductive performance (Jeuthe et al., 2015; Kurta et al., 2021). Understanding the sperm quality changes throughout the breeding period would aid the management of reproduction and selective breeding schemes in Arctic charr hatcheries.

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Acknowledgement

The authors acknowledge support from FORMAS under grant agreement 2018-00869 (NextGenCharr), the Kolarctic funding body under the ARCTAQUA project (grant agreement 4/2018/095/KO4058), and the Royal Swedish Agricultural Academy (KSLA, project no. VAT2021-0002).

INDUCTION OF SPERMATOGENESIS IN 1+ YEAR OLD GREATER AMBERJACK (*Seriola dumerili*) AFTER ADMINISTRATION OF SINGLE-CHAIN RECOMBINANT GONADOTROPINS

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Introduction

Greater amberjack (*Seriola dumerili*) begin to reach sexual maturity after 4 years, and the age can vary in different geographical locations (Kožul, Skaramuca, Glamuzina, Glavić, & Tutman, 2001). A long period of time is required, as only at five to six years of age all the population is considered fully mature. Therefore, shortening the time between generations by advancing the time of puberty might bolster future production (Levavi-Sivan, Bogerd, Mañanós, Gómez, & Lareyre, 2010; Molés et al., 2020)seabream, channel and African catfish, goldfish, eel, tilapia, zebrafish, Manchurian trout and Orange-spotted grouper. The hypothalamus in fishes exerts its regulation on the release of the GtHs via several neurohormones such as GnRH, dopamine, GABA, PACAP, IGF-I, norepinephrine, NPY, kisspeptin, leptin and ghrelin. In addition, gonadal steroids and peptides exert their effects on the gonadotropins either directly or via the hypothalamus. All these are discussed in detail in this review. In mammals, the biological activities of FSH and LH are directed to different gonadal target cells through the cell-specific expression of the FSH receptor (FSHR. We report, here, the *in vivo* efficacy of recombinant gonadotropin-based therapies in promoting gonadal development in 1+ year old, pre-pubertal greater amberjack.

Materials and Methods

An experiment was conducted to determine the minimum effective dose for the administration of recombinant *Seriola dumerili* single-chain gonadotropins (rsdGths), follicle-stimulating (rsdFsh), and luteinizing (rsdLh) hormones. Sixteen-month-old hatchery-produced F1 pre-pubertal male and female greater amberjack (October 2021) were treated with one of three doses of rsdFsh (Saline, 5, 10 or 15 μg kg^{-1}) or two doses of rsdLh (5 or 10 μg kg^{-1}) over six and two weeks, respectively. At the end of rsdFSH administration, gonads were dissected to estimate the gonado-somatic index (GSI) and for histological investigation. Blood samples were obtained from fish treated with rsdFsh as well as from those treated with rsdLh at regular interval to measure sex steroid hormones. In a second experiment, 1+ year old fish were injected with the best doses of rsdFSH and rsdLH established in the previous study, once a week over a period of twelve weeks, during the natural reproductive season for the species (March-June 2022). As above, blood samples were collected regularly to monitor variations in the plasma levels of sex steroids. A week after the last rsdGths administration, fish will be euthanized and gonads collected for the estimation of the gonado-somatic index (GSI) and for histological investigation.

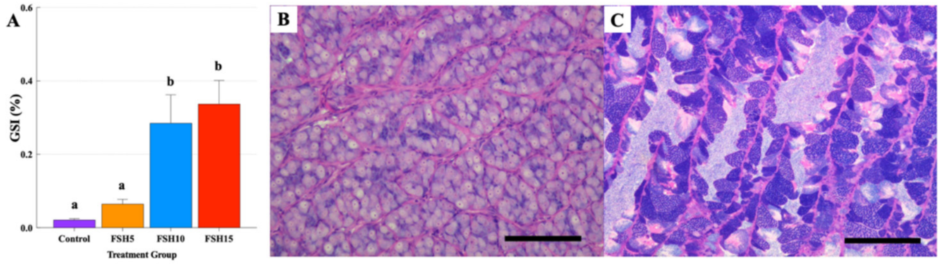

Fig. 1 Response to a sixweek administration of rsdFSH on hatchery-produced F1 immature male greater amberjack. A) Mean Gonado-somatic index (± SEM). B) Testicular sections dominated by spermatogonia. C) Testicular lobules filled with spermatozoa. The scale bar indicates 200 µm (B) and 500 µm (C).

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Results

In the Control group, the testes were dominated by spermatogonia, and spermatocytes represented a small fraction of the gonadal content. The administration of 10 and 15 μg kg^{-1} of rsdFsh stimulated proliferation and differentiation of germ cells and a significantly increased GSI values (Fig. 1). Sperm was found within the testes, and histology confirmed the presence of all spermatogenic stages and spermatozoa in the seminiferous lobules. Additionally, male plasma levels of testosterone (T) and 11-keto T differed among groups and with a significant increase after administration of 10 and 15 μg kg^{-1} of rsdFsh.

Contrary to the males, no differences were found in the GSI values of females between groups. However, the mean diameter of the largest primary oocytes differed significantly, with a 27 and 29% increased size in females treated with 10 and 15 μg kg^{-1}, respectively. Although ovarian lamellae filled with oogonia and primary oocytes were found in females from all groups, micrographs of ovarian sections displayed a higher content of oocytes in the perinuclear stage in the ovaries of females treated with 10 and 15 μg kg^{-1}. Significantly increased plasma levels of T were also found in females given 10 and 15 μg kg^{-1} of rsdFsh, but no differences were detected in the plasma levels of 17β-Estradiol. Finally, no differences were found in plasma sex steroid levels in fish after injection with rsdLh.

Collection of experimental data from the second experiment in underway, and the results will be presented at the conference.

Discussion

The administration of rsdFsh only, was effective to induce gonadal development and early maturation in pre-pubertal male greater amberjack. Although oogenesis was not achieved in females, the increase in mean diameter of primary oocytes indicates that a longer administration of recombinant Fsh would have been beneficial for further development of the gonads. All together these results demonstrated the biological potency of rsdFsh, and that it can be used to induce gonadal development in immature greater amberjack, therefore shortening the onset of puberty. The response to a combined usage of rsdLh and rsdFsh in plasma steroid levels and gonadal development is currently under examination, and based on the results, the appropriate doses and timing for administration will serve the purpose of implementing protocols for full maturation on pre-pubertal fish during the regular reproductive season.

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EFFECTS OF DIFFERENT RED SEA TEMPERATURES ON THE GROWTH, FEED CONVERSION RATIO (FCR) AND NUTRIENT UTILIZATION OF CULTURED GILTHEAD SEABREAM *Sparus aurata*

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Introduction

Temperature is considered to be one of the important environmental factors affecting growth in fish (Jobling, 1996). In this study, the gilthead seabream *Sparus aurata*, an aquaculture species that is of commercial importance in Saudi Arabia, was cultured at different Red Sea temperatures to determine its growth, FCR and nutrient utilization. The results obtained from this research will inform the growth performance and FCR of the fish grown in the Red Sea where the water surface temperature of the sea varies during summer and winter months.

Materials and Methods

Juvenile (212 ± 4 g) and pre-adult (492 ± 4 g) gilthead seabream *Sparus aurata* were cultured in separate experiments using 1-ton tanks supplied with seawater from the Red Sea wherein three different rearing temperatures were set as treatments (low: 24°C; mid: 28°C; high: 32°C). The fish were fed with a commercial diet (49% crude protein; 2.9% crude fat; 21.14 MJ kg^{-1} gross energy) to apparent satiation for 10 weeks. Fish were sampled every two weeks to determine growth and survival, while feed intake was determined daily. Whole body proximate composition of the fish was determined at the start and end of the culture trial.

Results

The results showed that growth was significantly higher at 24°C while lowest at 32°C in both the juvenile and preadult size fish. No significant difference on growth was observed between the 24 and 28°C group in the juvenile fish. However, in the pre-adult fish, the growth was significantly lower in the 28°C group than in the 24°C group. A significantly lower FCR value was observed at 24°C, followed by 28°C, while highest FCR was observed in the 32°C temperature in both fish sizes. Regression analysis suggests that SGR and feed conversion efficiency in the smaller fish were optimum at 25.9°C and 24.4°C, respectively, while in bigger fish at 24.8°C.

Protein efficiency ratio was significantly higher in the 24°C as compared to 28 and 32°C in both fish sizes. Body lipid, lipid retention and energy content in juvenile fish showed increasing trend as the temperature increases from 24 to 32°C, while body protein, protein retention and moisture content were significantly reduced as the temperature increases from 24°C to 32°C.

Discussion

Our results showed that better growth performance was observed at 24°C while the lowest growth was observed at 32°C in both the juvenile and pre-adult fish. Our data suggest that smaller size gilthead seabream has higher temperature tolerance range than bigger size gilthead seabream. The optimum temperature for growth observed in this study was between 25.9-24.8°C which is in line from previous report (26-24°C) (Hernández*, et al.*, 2003). As the temperature increases from 24°C to 32°C, the body lipid and energy content in juvenile fish showed increasing trend (fattier) while body protein, protein retention and moisture content were significantly reduced, suggesting that there was an increased utilization of protein over lipid to compensate energy demand at higher temperature. Nutritional manipulation in the diet by (slightly) increasing its protein level to compensate the protein needed by the fish for energy and growth at higher temperature conditions is suggested. The application of feed additives such as probiotics to improve overall status of the fish during temperature stress can be done.

Conclusion

The best temperature for growth of gilthead seabream cultured in the Red Sea was between 25.9-24.8°C while FCR was best at 24.8-24.4°C. Our results suggest that higher protein diet is recommended for gilthead seabream cultured at higher temperature conditions in order to support fish homeostasis and growth. Determining the optimum protein to energy ratio in the feed for gilthead seabream cultured at higher temperature conditions is suggested for an efficient and economical cage culture.

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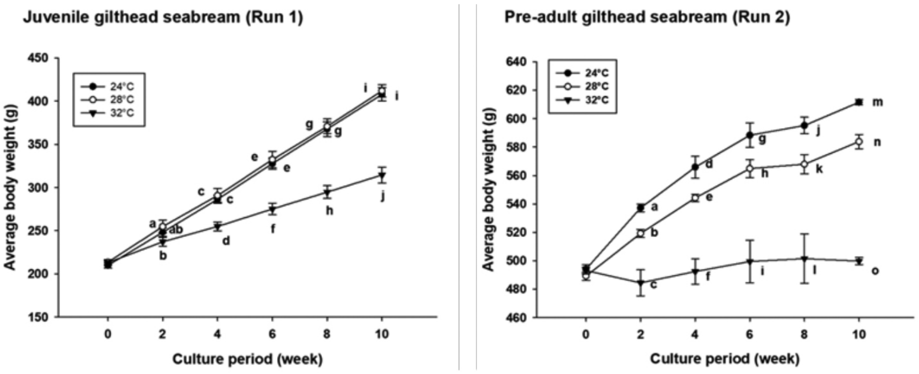

Figure 1. Average body weight of juvenile and pre-adult gilthead seabream cultured at different rearing temperature conditions (24, 28 32°C) for 10 weeks. Values represent means ± SEM (*n*=3). Different letters in a particular culture period indicate significant differences (*p*<0.05) between treatments.

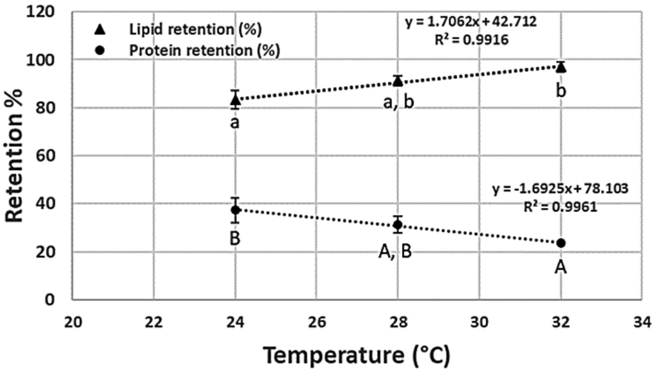

Figure 2. Correlation between temperature and protein retention (%) and lipid retention (%) of gilthead seabream juvenile. Values indicate means ± S.E.M. (n=3). Different letters indicate significant differences (p<0.05) between treatments.

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SMELLING THE DANGER OF CLIMATE CHANGE: THE CASE OF ATLANTIC SALMON OLFACTORY ORGAN

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Introduction

Recent studies have revealed the threats of climate change to aquaculture, an industry that has the potential to provide food and employment to the growing world population. To support further growth and expansion, the aquaculture industry must develop robust climate adaptation plans that are evidence-driven and supported by empirical data. This is particularly relevant for Atlantic salmon (Salmo salar), one of the major farmed fish in the world, where the impacts of future climate scenarios have already been identified by several prediction models. Nonetheless, biological relevance of these impacts is not yet fully elucidated.

The mucosal organs (i.e., gills, skin, olfactory organ, gut) of fish are highly sensitive to environmental changes. Their molecular and structural alterations often provide an indication of the quality of the environment; thus, these organs are promising models to study the impacts of climate-related stressors to fish health and welfare. In particular, the olfactory organ, which is a key organ for both immune defence and olfaction, has been shown to be highly affected by elevated near-future CO_2 levels via alteration of responses to sensory cues. In this study, we investigated how long period of elevated temperature and a heatwave, impacted the molecular and cellular properties of Atlantic salmon olfactory organ.

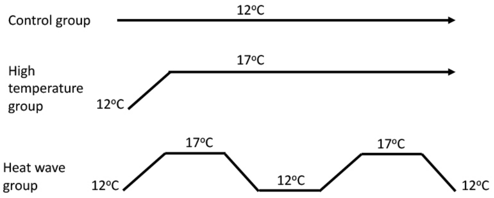
Materials and methods

A group of Atlantic salmon post-smolts were reared under three thermal conditions – Control group was at 12ºC, High temperature group was at 17ºC and Heat wave group was reared at 12ºC then gradually increased to 17ºC, then progressively lowered again to 12ºC. The experiment was carried out over 2 months. There were 2 rounds of heatwave episodes in the trial. The olfactory organs were collected at several time points for gene expression analysis and histology.

Moreover, olfactory organ explants from these groups of fish were developed at several time points during the trial and exposed to hydrogen peroxide to trigger oxidative stress.

Results and Discussion

Concerning responses to the increase of temperature, olfactory organ behaved as an immune tissue: the stressor stimulated expression of immune genes while several other functional groups were downregulated. This trend was seen in both groups (i.e. High and Heat wave groups) and in all time-points with exception of Heat wave group at the end of the first round. The immune responses were complex and include all tiers: pathogen recognition, communication and signalling via chemokines and cytokines, regulators, humoral and cellular effectors, development, and differentiation of lymphocytes. Increase was also observed in recently identified transcription modules (TM) of responses to inflammation, bacterial and viral infections. This was in parallel with the decreased abundance of erythrocyte markers (mainly haemoglobins transcripts) observed in five of eight comparisons, which suggested reduced circulation of blood in the olfactory organ. The greatest immune stimulation was found in the Heat wave group at the peak of the first wave and especially in high temperature (17°C) at the end of the second heatwave.



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Development of outgrowths from the olfactory explants were slower in the Heat wave group, especially at the end of the second wave. Inducing oxidative stress in the explants showed varying degrees of alterations, though considerable impacts in the ability to respond to the chemical stressor were identified during the second month of the thermal manipulation. During the first heat wave, all treatment groups showed almost similar tendency in the expression of a panel of antioxidant markers following oxidative stress induction. However, at the peak of the second heat wave, impairment of response to oxidative stress was identified especially in the Heat wave group. In particular, genes regulating the glutathione system did not respond to the oxidative stressor. Moreover, autophagy was induced in the High temperature and Heat wave groups after the end of the second heatwave.

Conclusion

The results reveal that the olfactory organ of Atlantic salmon was sensitive to climate-related stressors. Long period of high temperature and heatwave could alter the immune defences of the olfactory organ. Moreover, a heat wave likely impaired the ability of the olfactory mucosa to respond to oxidative stress.

Acknowledgements

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RISKS OF PERACETIC ACID-BASED DISINFECTANT TO ATLANTIC SALMON PARR REARED IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

There is a high regard for peracetic acid (PAA)-based disinfectants in recirculating aquaculture systems (RAS) because of the low risk of bioaccumulation, fast degradation with neutral residuals and minimal impact on biofilter performance. There is significant evidence regarding the physiological consequences of PAA in salmon post-smolts but not during the parr stage. Given that PAA efficacy is affected by different factors, it is a not advisable to extrapolate the health consequences in post-smolts to parr. Currently, the no-observed-effect concentration in Atlantic salmon parr is unknown. In addition, it remains to be evaluated how Atlantic salmon response to prolonged exposure to low dose PAA. The present study evaluated the effect of an acute and chronic PAA exposure on Atlantic salmon parr health and welfare.

Material and Methods

In this first trial, nine experimental RAS units were employed, where each unit was dedicated for one PAA concentration (0.0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L). Fish were exposed to the target PAA concentration in a static system for 1 h and the exposure protocol was repeated after a 52 h recovery. In the second trial, PAA was applied to Atlantic salmon parr reared in freshwater recirculating aquaculture systems over a 4-week period at a target concentration of 1 mg/L. Two administrations were tested, either in pulse (every 3 days) or continuous. The group that did not receive PAA served as a control. Fish tissue samples were collected for histology, gene expression, and biochemical analyses at day 0 and after 2 and 4 weeks of exposure.

Results

The fish survival was 100, 80 and 0%, respectively ≤ 1.6, 3.2 and 6.4 mg/L (Figure 1).

Fish swimming behaviour was normal in PAA ≤ 1.6 mg/L, whereas it become erratic with air gasping for PAA ≥ 3.2 mg/L. The fish appetite did not change among the different PAA treatment groups. Skin and gill histopathological alterations were pronounced in PAA ≥ 3.2 mg/L, characterized by a poorer skin condition and necrotic gill lamella (Figure 2). The skin acidic mucous cells density was 55% lower in the 6.4 mg/L group than the 0 mg/L group. The sub-lethal water pH values observed in the 6.4 mg/L group after PAA administration may have played a confounding and compounding factor to the PAA toxicity response in this group.

The expression of genes encoding for antioxidant defense in the olfactory organs, skin, and gills changed during the trial, but the time-related effects were more prominent than inter-treatment impacts. The antioxidant system in the mucosa of Atlantic salmon parr was responsive to PAA, corroborating earlier evidence in smolts. We found that the glutathione group of antioxidant genes was more susceptible to PAA. There was a tight regulation of mucosal and systemic oxidative stress as indicated by the profiles of reactive oxygen species and total antioxidant capacity in plasma and in skin mucus. Structural alterations have been identified in the gills, skin and olfactory organs.

Discussion

The current study identified the no-observed-effect concentration for PAA to be below 1.6 mg/L for Atlantic salmon parr and provided insights into its use as a water prophylactic strategy in RAS. Toxicity of PAA-based disinfectants is influenced by its acidified nature, which can interfere with the water pH of low alkalinity aquaculture systems.

The chronic exposure trial revealed that Atlantic salmon parr mobilised mucosal and systemic antioxidant defenses against the oxidative disinfectant PAA, but the mode of application did not impose a strong influence. The minimal effects of PAA application on the indicators of health and welfare of parr underscore the potential use of PAA as a routine disinfectant in recirculating aquaculture systems.

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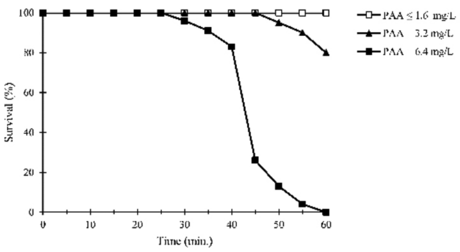

Figure 1. Percentage survival (%) of Atlantic salmon parr exposed to different PAA concentrations. (Nstart= 20 fish per tank).

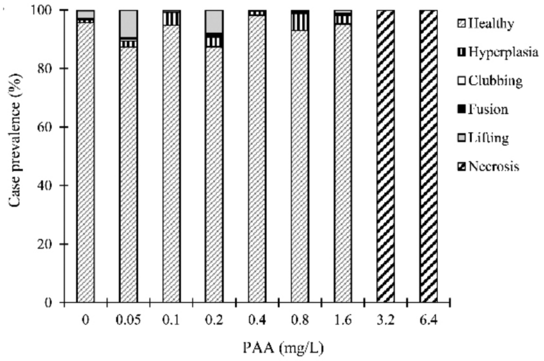

Figure 2. Histological assessment of gill health. A) Gill health was assessed by counting the prevalence of pathologies often observed following oxidant treatments in Atlantic salmon, including PAA exposure.

Acknowledgements

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MUCOSAL IMMUNOTOXICITY OF HYDROGEN SULPHIDE IN ATLANTIC SALMON SMOLTS

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Introduction

Hydrogen sulphide (H_2S) is a naturally occurring compound, which has received more attention in recent years due to its central role as gasotransmitter within many physiological systems, including teleosts. Endogenously generated H_2S controls several important metabolic functions, including angiogenesis, neuronal activity, vascular relaxation, glucose metabolism, energy production, and the inflammatory response. On the other hand, H_2S gas produced by anaerobic bacterial decomposition of protein and other sulphur-containing organic matter can be detrimental to the fish. This water-soluble gas has an identifiable odour of rotten eggs. In recent years, H_2S-related mortality has become a major challenge in saline recirculating aquaculture systems (RAS) of Atlantic salmon. Significant advances have been made in elucidating its formation in these systems, however, the biological processes underlying the adaptive responses of salmon to this molecule remains elusive. In this study, we focused on understanding the mucosal immunotoxicity of H_2S in Atlantic salmon.

Materials and methods

Acute exposure: Atlantic salmon smolts were exposed to low (0.6 µM/L) and high (1.2 µM/L) H_2S for 3 hours and thereafter allowed to recover for 24 hours. Unexposed fish served as the control. Mucosal organs and skin mucus were collected after the recovery period for gene expression analysis and metabolomics.

Chronic exposure: Atlantic salmon smolts reared in recirculating aquaculture system were exposed continuously to either 1 µg/L or 5 µg/L H_2S. The group without H_2S dosing served as the control. Tissue sampling was carried out pre-exposure and at 2-, 4- and 6-weeks post-exposure. To identify whether H_2S exposure affected the stress responses of salmon to a secondary stressor, fish were exposed to a confinement stress at the end of the exposure trial.

Results and Discussion

Acute exposure to H_2S exposure affected the expression of genes involved in sulphide detoxification, stress and immunity. The responses exhibited a concentration and tissue-specific manner. The olfactory organ and gills were behaving similarly towards H_2S. Structural alterations in the different mucosal organs were minimal, however, damage scores were higher in the high dose group.

The results of the chronic exposure trial are currently under processing and are expected to be ready before the meeting.

Conclusion

The results reveal that the immunotoxicity of H_2S in Atlantic salmon. These results offer insights into how fish interact with H_2S and are expected to contribute to addressing the issue of this toxic gas in salmon RAS-based farming.

Acknowledgements

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CHALLENGES AND OPPORTUNITIES IN THE APPLICATION OF THE 3R (REPLACEMENT, REDUCTION, REFINEMENT) PRINCIPLES IN AQUACULTURE RESEARCH: INSIGHTS FROM NORWAY

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Introduction

Excellent science and superior animal welfare go hand in hand. Therefore, ensuring that experimental animals are handled most humanely and ethically possible will significantly impact the scientific robustness of the data generated from a trial. The humane use of animals in scientific research is guided by the principles of the three Rs – REPLACEMENT, REDUCTION and REFINEMENT. Russell and Burch first described the 3R framework in their book The Principles of Humane Experimental Technique, first published in 1959 (WMS and RL., 1959). Since then, the concept has developed into an imperative component of research involving animals (Tannenbaum and Bennett, 2015).

In aquaculture research, the 3R framework has been applied for some time now, and the ethics boards granting licenses to conduct animal experimentation require that the concept will be involved in all aspects of the trial. This is highly relevant in Norway since at least 90% of the animals used in experiments in the country are fish (Norwegian Food Safety Authority or Mattilsynet, http://www.mattilsynet.no/fdu/om/arsrapporter). Therefore, there should be a continuous, upfront, and dynamic discussion from the different sectors, regardless of whether the involvement is direct or indirect in a trial, on how the 3R framework can be best utilised in aquaculture research. Norecopa is Norway's National Consensus Platform for advancing "the 3 Rs" in connection with animal experiments. Norecopa and Mattilsynet have been at the forefront of advancing the 3Rs in all species used for experimentation in Norway.

Unlike other animal models for research (e.g. rodents), you need to account for complex factors in handling aquatic animals. Aquaculture has changed dramatically in the last years (i.e. types of production systems, diversification of farmed species), which has also influenced how research is conducted. It is a must that the 3R framework adapts to this development. In order to achieve a broad, responsive, effective and innovative application of the 3R in aquaculture, sectors involved – from legislators down to the lab personnel - must venture into a learned discussion to identify the challenges and progress, as well as the opportunities that will define its continued relevance in the years to come.

This talk will highlight some of the challenges and opportunities in applying the 3R principles in aquaculture research, with a particular focus on Norway. We will highlight what had transcribed during the recently concluded National Workshop on 3Rs in Aquaculture Research which our group organised. In addition, we will discuss how the 3R principles are applied at different levels of our research at Nofima, especially our *in vitro* models and approaches to reduce the number of animals in fish health research.

Acknowledgements

This work was supported by the Norwegian Research Council projects 194050 (Insight) and 331680 (Aqua3R). We would like to acknowledge Norecopa, Norway's National Consensus Platform for the advancement of "the 3 Rs" (Replacement, Reduction, Refinement) for the 3R Prize in 2021.

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GROWTH-INHIBITION EFFECT OF *Phaeobacter inhibens* AGAINST VARIOUS STRAINS OF *Vibrio harveyi* ISOLATED FROM MARINE AQUACULTURE SPECIES

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Introduction

Vibrio harveyi is a Gram (-) pathogenic bacteria that causes high mortalities in temperate and tropical marine aquaculture affecting all developmental stages of important commercial farmed species including fish (e.g. European seabass, gilthead seabream), crustaceans (peneid shrimp) and shellfish (clam, oyster). Currently, there are no commercial vaccines available against *V. harveyi*. Autogenous vaccines are available but, due to the high variability and diversity of the *V. harveyi* group, the industry still mainly relies on the use of antimicrobials. As a consequence, there is an increasing number of *V. harveyi* field-isolates showing reduced susceptibility to the most used antimicrobials. Alternative methods are urgently required to control *V. harveyi* outbreaks in marine aquaculture systems.

The roseobacter *Phaeobacter inhibens* DSM 17395 has been previously evaluated as a safe probiotic candidate with antagonistic activity against pathogenic vibrios in marine aquaculture systems (Sonnenschein et al., 2021).

The study assessed the ability of *P. inhibens* DSM 17395 to inhibit the growth of *V. harveyi* isolates of distinct commercial origin, pathogenicity, serotypes and antibiotic resistance profile to estimate its potential contribution to the biocontrol of *V. harveyi*.

Materials and Methods

The candidate biological control agent *Phaeobacter inhibens* DSM17395 was tested against five distinct *Vibrio harveyi* isolates collected from the field (Table below).

Media

Reduced-carbon marine-broth (RCMB; sea salt 30 g.L^{-1}; bacteriological peptone 5 g.L^{-1}; yeast extracts 1g.L^{-1}); marine-broth (MB; sea salt 30 g.L^{-1}; bacteriological peptone 2,5 g.L^{-1}; yeast extracts 0.5 g.L^{-1})

Co-culture assays

Flasks containing 20 ml of RCMB were inoculated with 100 µl of overnight cultures of *P. inhibens* (~3x10^5 CFU/mL) and with a *V. harveyi* strain (~4x10^6 CFU/mL); or with *V. harveyi* only (Control). Co-cultures were incubated (22°C; 200 rpm) and samples taken after 1, 7 and 14 days of inoculation. Dilutions of the samples were plated onto vibrio-specific TCBS-agar (48h, 30°C) for *V. harveyi* enumeration.

Growth-inhibition assays with *P. inhibens* culture extract

P. inhibens cultures were extracted with ethyl acetate following standard extraction protocols, dried and resuspended in methanol. Various volumes of the extract were added to a multiwell plates and set aside until methanol evaporation. Then, 100 µl *V. harveyi* cultures (MB, OD_{600nm} ~ 0.1) were added to each well, the plates were incubated in a plate reader at 30°C and growth was assessed every 15 min by reading at OD_{600nm}. Growth curves were compared to those of the control cultures without *P. inhibens* extracts.

V. harveyi strain ID	Origin Host, Country, Stage	Clinical case	Antibiogram results
Aq 16-37	*D. labrax;* France; Hatchery	Yes	No resistance
Aq 23-64	*D. labrax;* France; Hatchery	Yes	AMX resistant
Aq 23-66	*S. aurata*; France; Hatchery	Yes	AMX resistant
Aq 20-88	*D. labrax;* Tunisia; Grow-out	Yes	AMX resistant
Aq 20-19	*S. aurata;* France; Grow-out	No	AMX resistant, FLU intermediate

D. labrax, Dicentrarchus labrax (European seabass); *S. aurata, Sparus aurata* (Gilthead seabream); AMX, Amoxicillin, FLU, Fluoroquinolone

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Results and Discussion

Co-culture assays **(Fig 1)**

In co-culture there was a clear inhibition of all the *V. harveyi* strains tested when grown with *P. inhibens*. This inhibition was significant 1-day after co-incubation and maintained throughout a long incubation period of 14 days. This indicates that the inhibition is not short lived nor overcome by the typically fast-growing *V. harveyi*.

Growth-inhibition assays with *P. inhibens* culture extract **(Fig 2)**

Growth inhibition was observed for all *V. harveyi* strains when incubated with crude ethyl acetate extract of *P. inhibens* culture, i.e. without *P. inhibens* cells or cellular fractions. *P. inhibens* produces secondary metabolite such as tropodithietic acid (TDA) and other bioactive compounds. TDA is described to have antagonising activity against other bacteria as well as quorum sensing properties.

Conclusion

Phaeobacter inhibens DSM 17395 significantly inhibited the growth of pathogenic *V. harveyi* isolated from distinct commercial origins, and this was at least partially mediated by *P. inhibens* extracts.

This biological control agent is a promising candidate to mitigate the propagation of *Vibrio harveyi* under aquaculture operations, as well as of other *vibrio* spp. of aquaculture significance as was previously documented.

P. inhibens DSM 17395 contribution to *V. harveyi* management should now be tested under commercial operations such as marine hatchery settings.

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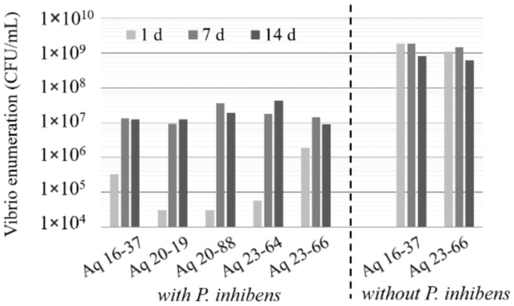

Fig 1: Time-series enumeration of *V. harveyi* isolates co-cultured with *P. inhibens*

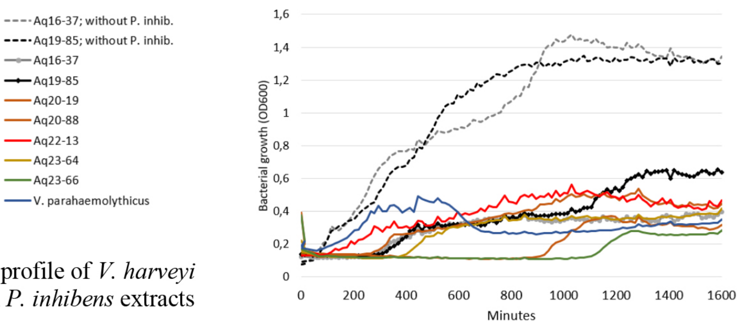

Fig 2: Growth profile of *V. harveyi* incubated with *P. inhibens* extracts

THE DOSE-RESPONSE OF SEAWATER ATLANTIC SALMON (*Salmo salar*) TO A MANNAN-RICH YEAST CELL WALL INGREDIENT TRADES BETWEEN GROWTH AND HEALTH BENEFITS

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Introduction

Preventive health is a pillar of modern aquaculture that functional nutrition cement. Mannan-rich yeast parietal fractions (MOS) are safe and natural ingredients widely documented to conditionally promote health, growth and/or feed performance across a diversity of farmed animals. To date, no MOS dose-studies are reported in aquatic species limiting the optimal application of this biotechnology. In Atlantic salmon, a specific commercial MOS product (sMOS) was recently confirmed to enhance gut and skin barrier functions (Micallef et al., 2017; Leclercq et al., 2020). The aim of this study was to characterize the dose-response relationship between growth, mucosal health and sMOS dietary intake with the view to inform the strategic use of this ingredient.

Materials and Methods

The 13-week trial tested 4 dietary groups in quadruplicate using Atlantic salmon post-smolt (173 ±3 g; 200 fish / tank; 740 L flow-through tanks; 16 ppt, 16L:8D). The test diets consisted of a “European” post-smolt baseline recipe (17% FM, 17% FO; 45% prot, 23% lip) supplemented pre-extrusion with sMOS (Lallemand SAS, France) at 0, 2, 4 or 6 kg/T (Control, 2-, 4-, 6-sMOS) and hand-fed to apparent appetite 4 times daily across the trial.

The trial (**Fig.1**) comprised an ideal (no challenges), a chronic heat-stress and a recovery period following exposure to hydrogen-peroxide (H_2O_2), together mimicking demanding commercial-conditions. Growth and feed performance were assessed, as well as gut and skin barriers (histomorphometry, skin mucus level and functionality) across sampling points (**Fig 1**). Difference to Control group were assessed using an unpaired t-test, significance was accepted at $P < 0.05$, data are shown as mean ± SEM.

Results and Discussion

There was a significant positive effect of 2-sMOS on SGR over the ideal (+9.6%) and f

In contrast to growth, the excretion of skin mucus overall increased with increasing sMOS intake (up to a significant +24.5% in 6-sMOS, **Fig 3**).

When considering mucus level per body-weight (BW) group and treatment (**Fig 4**), there was a significant negative linear relationship between skin mucus and BW in the Control (**Fig 4**); indicating weaker secretion in larger fish prioritizing for growth. In contrast, 2- and 4-sMOS appeared to remediate this apparent mucus deficiency in larger fish, while 6-sMOS further enhanced and standardized mucus excretion across BW-groups.

Further, an anecdotal reduction of mortality following H_2O_2-bath was recorded, from 3.47% in the Control and 2-sMOS groups to 1.39% in the 4- and 6-sMOS.

Modulation of nutritional markers, gut and skin cytoarchitecture as well as skin mucus properties in response to increasing dietary intake of sMOS will be presented.

Conclusion

Dietary sMOS elicited a growth and skin health benefit that is dose and body-size dependent. At low dose (2 kg/T), sMOS enhanced population growth and appeared to remediate the weak mucus excretion observed in faster growers. Higher intake (6 kg/t) further enhanced skin mucus excretion across body-sizes; without growth penalties but at the expense of the growth benefit observed at lower intake. This together suggests that the nutritional benefits achieved at low sMOS levels are increasingly traded towards health and resilience with increasing intake of this immune-modulatory compound.

These novel findings confirm the contribution of sMOS to overall farm performance and can be used to inform the strategic or seasonal use of this functional technology in support of targeted biomass build-up or mucosal robustness.

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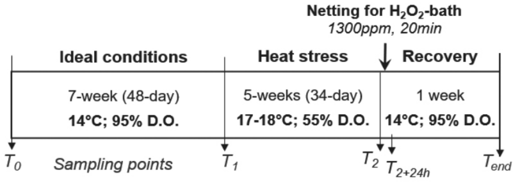


Fig 1: Experimental schedule

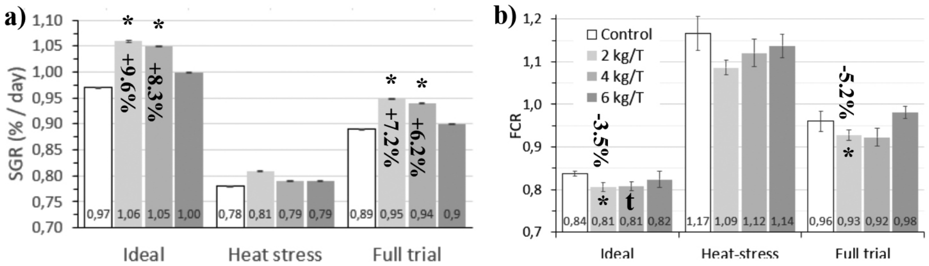
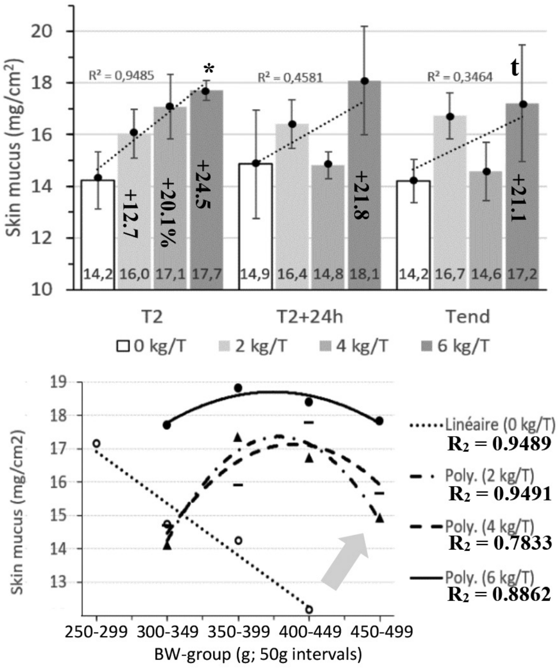


Fig 2 a) Specific Growth Rate and **b)** Feed Conversion Ratio. Mean ±SEM (n = 4)
Unpaired t-test showing difference to Control (*: P < 0.05; t (trend): P < 0.10)



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MITIGATION OF ATLANTIC SALMON WINTER-ULCER DISEASE (*Moritella viscosa*) USING A NOVEL YEAST-BASED FUNCTIONAL INGREDIENT

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Introduction

The Atlantic salmon winter-ulcer disease generates significant volume and value losses across the industry. Functional feed specifically targeting increased skin robustness and recovery from this pathology must be developed.

Prior studies have documented and part-deciphered the distinctive immune functionalities of contrasted inactivated yeast cell walls (YCW) products of different origins (e.g. species, strain, production process), biochemistry and morpho-functionality. In the Atlantic salmon, some YCW typologies promote, e.g., systemic immune-stimulation while others favor the skin immune barrier (Leclercq et al., 2020) or mechanical wound-healing (Leclercq et al., 2021). Based on this prior knowledge, a novel YCW product was pre- selected for its putative mitigation of infectious skin ulceration.

This study tested the capacity of a novel YCW product (nYCW) to mitigate and resolve a realistic Atlantic salmon winter-ulcer challenge combining exposure to a mild pathogen- load by immersion then to aggravating abiotic factors.

Materials and Methods

The trial lasted 7.5 weeks (102 days) using Atlantic salmon post-smolts (93 ± 4 g; flow- through tank; 80 fish/tank; 10.5°C, 22 to 28 ppt) challenged with the "typical" winter- ulcer agent (*Moritella viscosa*) by immersion at day 39. Abiotic manipulations were applied post-challenge to increase the disease severity or impact (27-day-post-challenge (dpc), temp. drop to 9.5°C; 36-dpc, salinity increase to 30-35 ppt; 63-dpc, 1 min. crowding) then to promote wound-recovery (63-dpc, temp. increase to 11°C).

Two test diets were tested in quadruplicate ($n = 4$) consisting of a base recipe (15% FM, 7% FO) either non-supplemented (Control) or supplemented pre-extrusion with the test ingredient (nYCW, Lallemand SAS, France) at 4 kg/T. Feed were hand-fed to apparent appetite 4 times daily over the trial's duration.

Survival profiles post-challenge were compared using a Mantel-Cox log-rank test. At 37, 50 and 63-dpc, all fish were anaesthetized for semi-quantitative wound-scoring based on worst wound-score (0: no wound; 1: focal scale-loss; 2: cutaneous lesion; 3: muscle exposed; 4: muscle exposed over Ø ≥ 1cm; score 2 to 4 marked either "a" (active) or "r" (recovering) based on wound-edge appearance; R: fully recovered wound(s) only).

Proportion of wound-categories were compared between diets within time-points (Kruskal-Wallis with Dunn's post-hoc tests). Gut and skin histomorphometry, and skin mucus functionality were assessed pre-challenge and at end-point (5 fish/tank/time-point). Data shown as mean ± SEM; significance accepted at $P < 0.05$.

Results and Discussion

Survival post-challenge (**Fig 1**) was significantly higher in the nYCW group showing, in particular, higher resilience following abiotic manipulation and crowding. Population mean-wound score was significantly improved in the nYCW group at all time-points (28%, 21%, 34% improvement compared to Control at 37, 50, 63-dpc respectively). At 37-dpc, 37% of fish had at least one open-wound (score 2 or 3) in the nYCW compared to 50% in the Control-group; followed by 28% and 41% respectively at 50-dpc when score 4 were first observed. Recovering wound were also first observed at 50-dpc and were significantly more prevalent in the nYCW compared to the Control (14.5 ± 0.6 % and 7.4 ± 0.8% respectively, not shown) at that time-point.

At the end of the trial (63-dpc), the proportion of fish showing no or only fully recovered wounds (scores 0 and R) was significantly higher in the nYCW compared to the Control (44.9 ± 6.8% and 20.9 ± 2.0% respectively; **Fig 2a**). Amongst wounded specimens, a higher proportion of fish had at least one recovering wound in the nYCW compared to Control group (51.9 ± 7.2% and 36.6 ± 7.6% respectively; trend, $P < 0.10$; **Fig 2b**).

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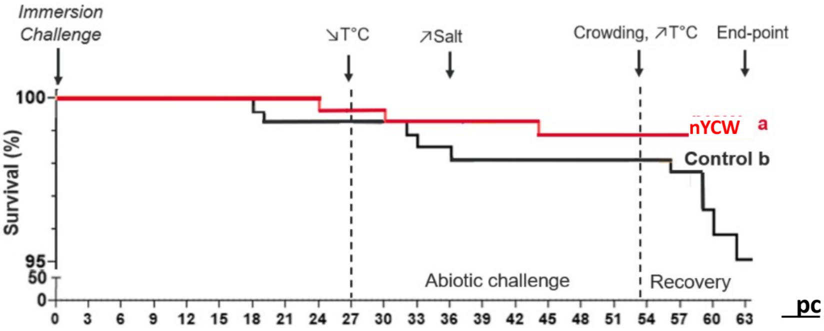

Fig 1. Survival profile following M. viscosa bath-challenge and abiotic manipulations (n = 4; Mantel-cox log-rank test; ^{a,b}: P < 0.05)

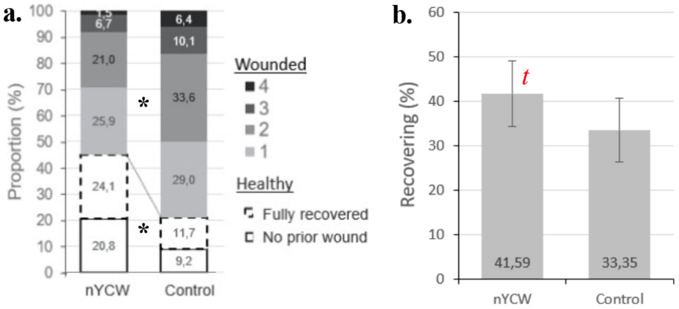

Fig 2. a. Wound-score distribution and **b.** Proportion of wounded fish with at least one recovering wound. Mean ±SEM; *: P < 0.05; ***t***: P < 0.10CV

Further data on gut and skin cytoarchitecture and skin mucus properties will be presented.

Conclusion

Combining a mild *M. viscosa* exposure by immersion with abiotic manipulations induced low mortalities but severe wound development and subsequent wound-recovery, together providing a realistic, commercially relevant winter-ulcer challenge.

Prolonged supplementation with a novel functional ingredient, *i.e.* 5 weeks prior and across the disease period, mitigated the severity of the winter ulcer outbreak and promoted healing of infectious skin ulcers. This ingredient has the potential to contribute to the preventive management of infectious skin diseases in the aquaculture industry.

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EFFECTS OF DIETARY FATTY ACIDS AND CONDITION FACTOR ON BALLAN WRASSE WELFARE DURING COLD WINTER TEMPERATURES

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Introduction

The deployment of cleaner fish is a widely used approach as biological control of the ectoparasitic salmon louse (Lepeophtheirus salmonis), one of the major challenges for the salmon aquaculture industry (Torrissen et al., 2013). However, concerns have been raised about the performance and welfare of cleaner fish under salmon sea cage conditions (Brooker et al., 2018). The two primarily used species, lumpfish (Cyclopterus lumpus) and ballan wrasse (Labrus bergylta) show distinct differences in their biology, life history and thermal tolerance that need to be addressed in husbandry practices and their use in sea cages. Ballan wrasse are known to remain in deeper and warmer waters while entering a dormant state with low activity and low metabolic rates in cold periods (Morel et al., 2013; Yuen et al., 2019). The aim of the current study was to evaluate the effects of enhanced dietary fatty acids and differences in condition factor on performance, welfare, and stress parameters of ballan wrasse reared at two different temperatures.

Materials and Methods

The experimental set-up is shown in fig. 1. During the feeding trial, ballan wrasse were fed with two different diets varying in essential polyunsaturated fatty acid levels (PUFA) in triplicated tanks, respectively. After three months, fish were sampled for chemical and fatty acid composition as well as welfare scoring, while the remaining fish were pit-tagged, and condition factor (CF) registered. Fish were distributed into six new tanks, each tank receiving similar numbers of fish from each diet and with high and low CF. During the temperature trial, three of the tanks were gradually lowered to 6 °C, while the remaining tanks continued at 15 °C. Analyses included mortality, growth and performance, chemical composition, fatty acid composition, morphological welfare scoring, gene expression in liver tissue, and skin histology.

Results

Dietary regimes had profound influence on the fatty acid profile of the fish without affecting growth and performance during the following temperature trial. There was a tendency towards less scale loss in fish fed with enhanced PUFA levels. This might indicate improved skin quality due to high n-3 PUFA levels, which has been previously shown for salmon (Berge et al., 2019). In the second part of the experiment, temperature strongly affected the performance of ballan wrasse. Fish reared at 15 °C showed higher growth rates, more fat and energy reserves and less ash content. On the other hand, fish reared at 6 °C showed negative growth rates, burned higher amounts of fatty acids and had very little energy reserves left at the end of the temperature trial. Concomitant gene expression analyses showed the positive effect of higher temperature on growth markers by up-regulating *GHrα* and downregulating *mstn* in fish reared at 15 °C compared to 6 °C. Moreover, two genes involved synthesis and oxidation of FAs (*elovl5, cpt1*) were upregulated in fish reared at 6 °C compared to 15 °C. Also, fish with high CF showed higher survival, growth rates and performance compared to fish with low CF.

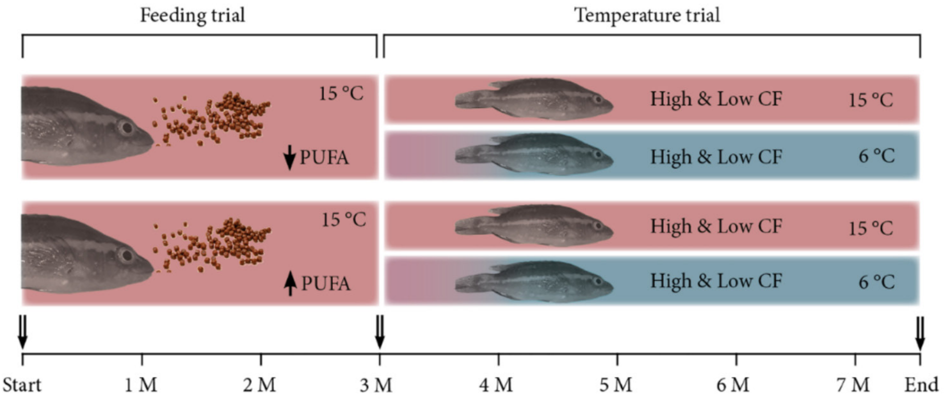

Fig. 1. Experimental design consisting of feeding and temperature trial. The timeline is indicating the start and end as well as the experimental duration in months (M). Errors indicate sampling points.

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Furthermore, temperature and CF affected the welfare of ballan wrasse. External welfare scoring showed higher prevalence and severity in emaciation, scale loss and the sum index score (sum of all measured welfare parameters) in fish reared at cold temperatures compared to fish reared at 15°C. Moreover, better welfare scores were found in fish with high CF, indicating those fish were coping better with low temperatures compared to fish with low CF. Fish reared at 6 °C also showed upregulated levels of *il-6* compared to fish at 15 °C, suggesting an enhanced immune reaction in response to cold water temperatures. Lastly, skin histology was assessed for the first time in ballan wrasse in the current study. Here, findings on epidermis thickness, as well as amount and organization of mucous cells showed that fish reared at 15 °C appeared to have better skin quality, while fish reared at 6°C showed signs of stress based on previous experience from skin histology on other teleost species.

Discussion and conclusion

Overall, low temperatures showed profound effects on the performance and external as well as internal welfare parameters of ballan wrasse. Thus, it can be considered a stressor for this species that will likely affect the delousing efficacy during winter. Results further showed that CF is an important factor for ballan wrasse to cope with low winter temperatures. These findings should be considered in the future use of ballan wrasse in salmon cages during winter months and supports the seasonal use of different cleaner fish species. If ballan wrasse are to be kept in sea cages during winter, enhanced feeding for increased storage of fat reserves is recommended. Moreover, assessment of the condition of cleaner fish before deployment is important to ensure sufficient energy resources.

The experiment was funded by the Norwegian Seafood Research Fund (FHF) as part of the project OptiRens (#901563).

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INFLUENCE OF PROBIOTIC *(Lactobacillus plantarum)* ADDITIVE IN FEED ON HEALTH STATUS OF RAINBOW TROUT *(Oncorhynchus mykiss)* AFTER EXPERIMENTAL CHALLENGE WITH *Aeromonas salmonicida*

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Nowadays, bacterial diseases are among the major issues of intensive aquaculture. Most infectious bacterial diseases can be controlled by treatment with chemotherapeutic agents, however, these pose a potential hazard to the environment and may also have a negative impact on the quality of the final product and human health. Apart from reducing the introduction of pathogens into the system and providing good living conditions, a number of substances that improve the health of fish are used, such as immunostimulants, probiotics, prebiotics, symbiotics and others (Palíková et al., 2015). Probiotics are microorganisms beneficial to the health of their host that actively inhibit the rate of colonization of the digestive tract by potential pathogens and/or stimulate the host‘s immune response. Bacteria usually do not colonize the intestinal mucosa of fish permanently, but must be continuously supplied to the fish in feed (Autin, 2010). One of the important representatives of the probiotic bacteria is the bacteria of the genus *Lactobacillus* (Balcazar et al., 2009). The effect of allochthonous lactobacilli was verified *in vitro* in a study conducted by Maruščáková et al. (2019). The tested probiotic strain reduced inflammatory changes after *A. salmonicida* infection through decreased expression of pro-inflammatory cytokines and increased expression of anti-inflammatory cytokines (Fečkaninová et al., 2019; Cingelová Maruščáková et al., 2021).

In our experiment we tested the effects of probiotic in the feed on haematological, biochemical and immunological parameters of rainbow trout (*Oncorhynchus mykiss*) as well as on fish ability to resist bacterial infection. The fish were divided into 3 groups: Group 1 was fed a feed with probiotic supplement (bacterial cells of *L. plantarum* R2 Biocenol™ in starch hydrogel layer) for 11 weeks, in group 2, pulsed-feeding was used (4 weeks of probiotic-supplemented feed, 3 weeks of probiotic-free feed and 4 weeks of probiotic-supplemented feed). Group 3 (control) was fed with probiotic free feed throughout the whole experiment (11 weeks). The first sampling was performed 7 weeks after the beginning of the test. On the same day, the remaining fish from all groups including control were infected with *Aeromonas salmonicida*, strain 89409 by immersion. The second and third sampling was done 1 week and 3 weeks after experimental infection, respectively. Blood samples were taken and used for analyses of haematological, biochemical and immunological parameters. Skin mucus samples were collected to determine the level of lysozym.

Mortality, changes of fish behavior and feed intake were monitored throughout the whole experiment.

The aim of the experiment is to verify the effect of probiotic applied in two different feeding regimes on health status of rainbow trout (*Oncorhynchus mykiss*) challenged with *Aeromonas salmonicida*. If positive effects are confirmed, the tested probiotic could be used for preventive treatment of the fish in intensive aquaculture systems and it could reduce the need to use antibiotics for the treatment of bacterial fish diseases.

This study was funded by the Internal Grant Agency of the University of Veterinary Sciences Brno (project no. 221/2022/FVHE).

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START-FEEDING OF WOLFFISH LARVAE USING DIFFERENT LEVEL OF SHRIMP AND SQUID AS CHEMO-ATTRACTANTS

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Introduction

The spotted wolffish (*Anarhichas minor*), is a cold-water teleost that has been identified as a promising candidate for cold-water aquaculture. Wolffish have the advantage of being well developed at hatching, allowing direct weaning onto dry feeds in artificial settings. However, the nutritional requirements are yet to be fully established in its different life stages. The Early life stages of finfish aquaculture represents a crucial bottle neck for production in terms of manual labour, feed costs and survival. Furthermore, the economic and environmental sustainability of the emerging aquaculture sector for this species will depend on the quality of ingredients utilised in future feeds. The challenge of fishmeal replacement with other high quality alternative ingredients for larval stages needs to be assessed for effects on first-feeding behaviour, growth, and survival. To this end, the present study aimed to investigate the potential of two formulated experimental diets (Fishmeal based *vs*. High quality marine ingredients) for differences between first feeding behaviour, growth and survival of Spotted wolffish larvae in a four-week feeding trial.

Materials and methods

Eggs of spotted wolffish (obtained from Akvaplan Niva, Tromsø, Norway) were fertilized in December 2021, incubated over several months at X degress and hatched at Mørkvedbukta research station at Nord University in July 2022 (Bodø). Shortly after hatching, two experimental groups (comparing two test diets) were established containing 700 wolfish larvae per tank (4 tanks/group). The experimental diets were formulated to be isonitrogenous (60% protein), isocalorific (20.3 MJ/Kg) and isolipophilic (12.5% crude fat) but composed of different protein sources. Diet 1 contained 45% fishmeal,10% fish protein concentrate and 5% squidmeal, krillmeal and shrimp powder respectively; whereas diet 2 contained 25% fishmeal, 10% fish protein concentrate 15% squidmeal and krillmeal and 10% shrimp powder. The experiment lasted for four weeks, starting July 18th and was terminated on August 15th 2022. Experimental groups were kept under continuous light and relatively constant temperature (xxx). The experimental diets were provided throughout the study at regular time intervals, 7 rations a day between 08:00 – 23:00. Survival was monitored on a daily basis and samples for biometrics, proximate composition, digestive enzyme activity and muscle histology were taken once a week during the experimental period.

Results

Preliminary results reveal Differences between the two diets for growth and survival, suggesting that replacing fishmeal with more expensive but higher quality marine ingredients needs to be assessed for trade-offs between feed costs against welfare, growth and survival on the economic an environmental sustainability of the emerging spotted wolffish aquaculture sector. The results of this study will be presented in more detail on the poster. Project is being co-funded by the Kolarctic CBC program and Nordland County.

WOMEN EMPOWERMENT AND GENDER MAINSTREAMING IN SUSTAINABLE AQUACULTURE

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Women represent 70% of the global aquaculture workforce, nevertheless, the great majority of management of the production is undertaken by men. The lack of comprehensive data on women in aquaculture and gender-oriented training is one reason why women are invisible in aquaculture policy. Empowering women in the aquaculture sector through awareness and specific training activities designed to promote the role of women in aquaculture and increase its visibility in the sector is one of the goals of the ASTRAL project.

ASTRAL (*All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture*) is a Horizon 2020 project focused on integrated multi-trophic aquaculture (IMTA) farming, 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 (fertilisers and feed) for another species.

Within the Atlantic region, we continuously organise awareness and training activities to promote not only sustainable aquaculture but also women's empowerment and gender mainstreaming with the goal for these initiatives to continue beyond the project.

All-Atlantic Pledge Campaign (2021)
ASTRAL submitted a pledge at the All-Atlantic Pledging Campaign which aimed to engage with other stakeholders, strengthen collaboration, promote partnerships, mobilise partnerships and establish dialogues.

Training on waste upcycling and women empowerment (2021)
During World Ocean Day in 2021, a several activities were organised in Makoko fishing community of Lagos State in Nigeria, including a training session targeted to women.

Networking Friday on Women Empowerment in Aquaculture (2022)
As a celebration of the International Women's Day, a networking online session was held. The panel was filled with brilliant women in aquaculture working on the most diverse sectors, from international NGO (WWF Africa) to business and industry (Atlantic Sea Farms, EUA) as well as academia (Federal University of Pará, Brazil) and network (Women in Scottish Aquaculture (WISA), Scotland and Catfish Farmers Association of Nigeria) organizations. The session was moderated by a young researcher and Ocean Youth Ambassador at University Cape Town, South Africa.

Sensitisation and Women Mainstreaming into Aquaculture in Nigeria (2022)

A workshop was organised in Nigeria to support women empowerment in aquaculture. This workshop supported women involvement in aquaculture by promoting their entry into new and emerging markets and profitable enterprises/businesses in EU and non-EU Atlantic countries.

ASTRAL continues to work towards promoting sustainable aquaculture, food security and improved nutrition as well as promoting gender quality and empowering women and girls. As a result, we are also contributing to reaching SDG 2 (Zero Hunger), 5 (Gender Equality) and 14 (Life Below Water). Further information at www.astral-project.eu/

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THEME:
THE OCEAN:
LIFE AND LIVELIHOODS
VENUE: MAKOKO COMMUNITY, LAGOS || TIME: 9:00AM
PARTNERS:


NETWORKING FRIDAY on
Women Empowerment in Aquaculture
March 4, 2022. 1-3 PM UTC - http://aircent.re/netfridays
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European Union All Atlantic Ocean
Sustainable Profitable and Resilient Aquaculture (ASTRAL)
Organizes
ONE - DAY SENSITIZATION WORKSHOP FOR WOMEN
MAINSTREAMING INTO AQUACULTURE IN NIGERIA
VENUE: NIOMR CONFERENCE ROOM

A NUTRITIONAL BIOENERGETICS MODEL: FISH GROWTH, OXYGEN CONSUMPTION AND WASTE PRODUCTION IN RESPONSE TO FOOD COMPOSITION

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Introduction

Nutrition is of prime interest in finfish aquaculture and plays a critical role not only because it influences production costs but also because it affects fish growth and waste production. Protein is the forefront of finfish nutritional research as fish have high dietary protein requirements. In addition, nitrogen derived from protein catabolism is one of the main water pollutants in recirculating aquaculture. The quantitative study of the energy and nutrients entering the organism via food and its partitioning into various metabolic processes, is one of the main goals of many experiments with fish. Such experiments usually involve extensive use of laboratory facilities for long periods, which make, from both ethical (3Rs) and cost perspectives, mathematical models important tools for designing and planning of scientific experiments.

The nutritional bioenergetics model we propose is based on an ensemble of rules that describe the processes of digestion, absorption and the allocation of energy derived from food to metabolic processes of growth, maintenance, maturation, and reproduction according to a set of priority rules. The model is based on the Dynamic Energy Budget (DEB) theory, a qualitative and quantitative framework to study individual metabolism throughout the entire life cycle of an organism making explicit use of energy and mass balances (Kooijman, 2010).

Material and Methods

The model is an extension of the standard DEB model (Sousa et al., 2008; Kooijman, 2010) and assumes three life stages (larvae, juvenile and adult) as well as metabolic accelerated development for early stages which is an established practice for studying fish species in the DEB context (Kooijman, 2014; Lika *et al.,* 2014, Stavrakidis-Zachou, 2019). Additionally, the model incorporates a digestion-assimilation module to simulate the food dynamics in the gut and the process of assimilation of the macronutrients from the gut wall. The model allows to track the fate of nitrogenous waste. The conceptual organization of metabolism is presented in Figure 1.

The model was parametrized for three fish species (*Sparus aurata*, *Salmo salar*, and *Oncorhynchus mykiss*) using commonly available data for fish growth, feeding, reproduction and gastric evacuation time. The model was validated using published data on weight, oxygen consumption, carbon dioxide production and total ammonia nitrogen (TAN) excretion for the three species reared in a range of temperatures and under different food compositions.

Results

Overall, the model performed well and was able to capture the diverse nature of the inputs of the validation datasets. On average the model predicted better the body weight than gaseous exchange and nitrogenous waste. Results of this study show that growth is strongly linked with the amount of feed consumed where a higher ration results in higher weight gain. Moreover, diets rich in protein translate in high production of Total Ammonia Nitrogen while an increase in feeding frequency can result in lower daily fluctuations of gas exchanges.

An emerging property of the model is that it captures the effects of food composition on assimilation, which in essence translates to the effects of protein-energy (PE) ratio in the diet. Food either low or high in protein, results in low assimilation rate. It follows, that for a given ratio of fats and carbohydrates, there exists a specific protein fraction where assimilation is maximized. The protein fraction that maximizes assimilation as well as the maximum value depends on the fat to carbohydrate ratio and, thus, on energy content.

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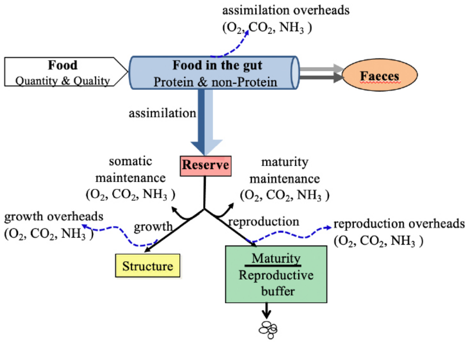

Figure 1. Conceptual representation of the metabolic processes. Food is partitioned into protein and non-protein components. Energy assimilated from food is added into reserves and subsequently allocated to fuel the metabolic processes: A fixed fraction ϰ of the mobilized flux is allocated to somatic maintenance and growth and the remaining (1−ϰ) to increase and maintain maturity or to reproduction.

Conclusions

We have developed a mechanistic model that focus on both energy and nutrient fluxes. The approach followed allowed to model the bioenergetics of fish throughout their life cycle as a function of temperature, food quantity and quality, and feeding frequency. The model allows simulations of growth, feeding, oxygen consumption, carbohydrate production, Total Ammonia Nitrogen, and solids, with hourly resolution. The model is one of the main components in the AQUAEXCEL^{2020} virtual laboratory (https://ae2020virtuallab.sintef.no/), which has been developed to enable virtual experiments in aquaculture research facilities.

Acknowledgements

The study was financially supported by the European Union's Horizon 2020 research and innovation program under grant agreement No. 652831 (AQUAEXCEL2020).

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RESPONSE TO SOCIETAL CHANGE AND CRISES. LESSONS LEARNED BY THE NORWEGIAN AQUACULTURE INDUSTRY IN 2020-2022

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Introduction

Norway is the world's second largest exporter of fish and seafood. Since 2019, the export value has surpassed 100 billion NOK. The high degree of export implies dependency between producing and consuming countries. Over the years, the Norwegian seafood industry has experienced several demand and supply shocks, probably influencing how the industry responds to new crises.

In March 2020, a national lock-down due to the Covid-19 pandemic was issued. Value chains related to food production were categorised as critical for society, meaning primary production and processing plants could keep their operation going. Still, outbreaks of the virus had to be handled. In January 2021, closed borders in the middle of the annual cod fisheries raised concern as foreign employees could not enter the country.

As a part of the project "*Seavid19 Ensuring economic sustainability in the seafood industry during crisis*" financed by the Research Council of Norway (grant no. 326647), researchers and industry actors collaborate to study effects and consequences of Covid-19 for the Norwegian seafood industry. Looking at different stages of the pandemic, the response to the pandemic, including effects of governmental strategies, challenges, opportunities and changes, are explored. This knowledge adds to previous studies of Covid-19 impacts in the seafood sector (Love et al. 2021).

Materials and methods

Interviews with 18 persons working in different parts of the seafood industry provide data for the study. This includes actors located in different parts of the country, belonging to the value chains of aquaculture (salmon) and wild fisheries (white fish, pelagic fish, and shellfish). Interviews focused on challenges and opportunities, which types of measures the authorities implemented and the consequences of them, as well as changes or innovations related to the pandemic. In sum, interviews provided learning about the industries' crises handling and potential to handle further crises.

Results

In March 2020, the Norwegian Seafood Association expressed concern about potential consequences for the seafood industry. Reduced capacity on planes affecting transport of seafood was one of the reasons for their worry. Measures such as allowing increased production for farmed salmon and the opportunity to transfer wild fish quotas from one year to the next were suggested.

Defining food production as critical for society was a key government strategy. This meant that schools and kindergartens remained open for children of employees in these industries, and production could be maintained if regulations for contamination were followed. The Norwegian Ministry of Trade, Industry and Fisheries and the trade organisations established regular digital meetings to discuss the current situation for the seafood actors. From the industry's point of view, this was decisive to get through the pandemic.

The interviewed salmon producers experienced both opportunities and challenges during the pandemic. With a large share of the Norwegian salmon being transported with passenger flights, the exporters experienced several challenges when this disappeared almost overnight. Only cargo planes had the possibility to transport seafood to the markets and with other export items competing for the same space, freight rates became very high. However, the market adapted with lower purchase prices and higher sales prices. At the same time, Norwegian salmon experienced other difficulties as well. China introduced double packaging in the beginning of 2022, following a burst of Covid-19 cases in Beijing.

Covid-19 also led to changes in consumer behaviour and commodity flows. While the HoReCa-market was hit hard, supermarkets, online shopping and take-away experienced a large increase, due to national lockdown or people being in quarantine. For the salmon exporters, increased demand from supermarkets has largely compensated for the decline in demand from other customer segments – such as HoReCa. The industry had to restructure – heavily focusing on global marketing and e-commerce.

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Interviews showed that the aquaculture industry, who operate in global industries, are used to adapting to change. Compared to other industries, like tourism (Škare, Soriano & Porada- Rochoń, 2021) or cultural and creative sectors (Travkina & Sacco, 2020), the seafood industry did not require significant public support measures.

Conclusion

Export figures show that the Norwegian salmon aquaculture managed to adjust very well to the changes caused by Covid-19. The strategies applied by the government were important for maintaining production throughout the different stages of the pandemic. As with previous experiences, the Norwegian government and seafood industry has gained knowledge that will be valuable in situations of rapid change or crisis in the future.

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INTELLIGENT MONITORING SYSTEM FOR INDOORAQUACULTURE TANKS

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Introduction

Global fish consumption has increased at an average annual rate of 3.1% from 1961 to 2017, which ranks higher than all other animal protein foods (meat, dairy, milk, etc.) That puts tremendous pressure on marine ecosystems, as it equates to roughly 180 million tons of fish species being consumed each year [1]. The aquaculture and fish-farming industry has been gaining traction as acommercially viable, sustainable and eco-friendly path forward.

In aquaculture explorations, fish and other aquatic species are bred, nurtured, harvested, and pro-cessed under controlled conditions designed to maximize quality and yield and minimize costs and environmental impacts. Traditionally, indoor aquaculture tanks rely heavily on periodic human inspection and operation. Technological innovation brought forward by aquaculture engineer- ing comes in response to the demand for even tighter control of water quality parameters, more efficient monitoring of fish larvae growth, and more automated "intelligent" feeding processes.

The most studied tasks requiring automation typically revolve around detecting fish size charac-teristics [2] (i.e., length, area, width and/or perimeter), fish counting or direct biomass estimation.Biological models can then be used to derive total fish mass from a single measure [3]. Lopes et al. [4][5] expanded on this notion, developing a robotic system equipped with a camera and two laser projectors capable of directly estimating in real-time the biomass present in a large fish tank,with between 10% and 17% of relative error in biomass volume in a real aquaculture environment.

When it comes to fish biomass estimation in specific, estimates are crucial for evaluating fish growth during its growth cycle in the hatchery/nursery stages. The statistical characterization of a given's tank population is fundamental for aquaculture explorations to control and adjust food and medicine dosage, to be able to detect fish population loss in early stages, and perhaps most importantly, to adequately monitor growth rates to decide the best timeline for financial decisions.Further advancement of economies of scale in industrial aquaculture nursery/hatchery scenarios are thus heavily dependent on the automation of monitoring and quality assurance processes.

To address this problem, this paper will present the Feedfirst Intelligent Monitoring System for Indoor Aquaculture Tanks, a system developed hand-in-hand with industry partners designed withthree goals in mind: (i) continuous monitoring of water quality; (ii) continuous monitoring of larvae growth and population size; and (iii) automation and optimization of daily feeding.

Feedfirst Intelligent Monitoring System

The Feedfirst system was tailored to industry partners requirements in indoor aquaculture scenar- ios and the ultimate design goal is to close the perception-actuation loop, i.e. automate feeding control in accordance to perceived fish growth parameters. All measures from water quality to image data are relayed to a central computer that is responsible for both processing the incomingdata and providing a user-interface to the tank operator (see fig.3).

The machine vision processing pipeline is intended to provide two important metrics: the count ofpopulation size and the size of each larvae fish. To achieve this, the submerged cylinder possesses2 stereo-mounted cameras synced with an illumination system. Red light wavelengths are used so as to keep environmental disturbance to a minimum. Acquired images are subject to an imageprocessing pipeline as shown in fig. 2. The prototype system was tested in operational conditionsin a indoor aquaculture in Portugal.

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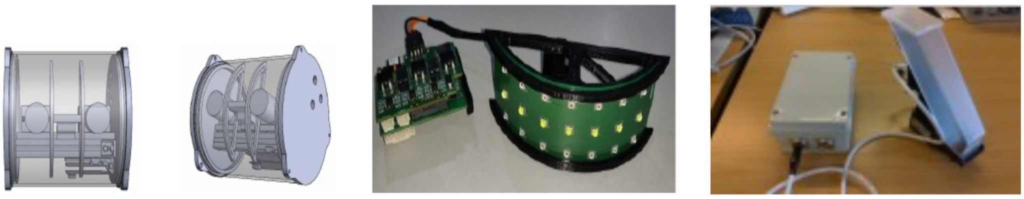

Figure 1: Left image shows the design of the 150x150 mm watertight cylinder capable of withstanding pressure up to 1 bar; The next images show the structured light illumination system and the feeder controller hardware. A truly "intelligent" system entails translating the knowledge from the perception of larvae growth to automating feeding control

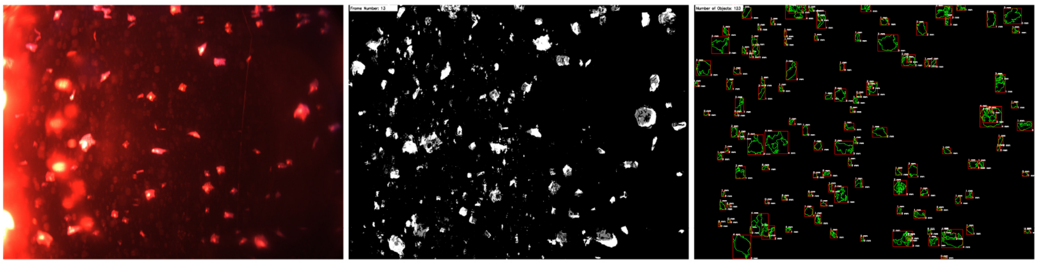

Figure 2: Computer Vision processing pipeline: from the acquired raw image, a background subtraction and blob detection algorithm and subsequent filtering provide both the count of detected larvae and its size.

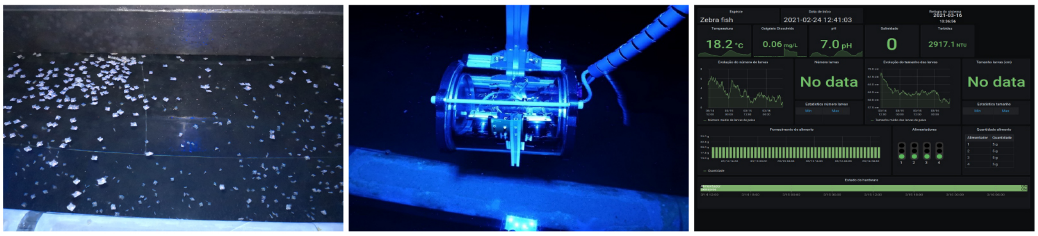

Figure 3: The first two images show the Feedfirst system in operational field test scenario of indoor fish-farming of turbot larvae. The image on the right shows the user interface that is displayed to the operator.

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A BIOENERGETIC MODEL FOR GILTHEAD SEABREAM (*Sparus aurata*) AND EUROPEAN SEABASS (*Dicentrarchus labrax*) GROWTH AND METABOLISM: THE EFFECT OF DISSOLVED OXYGEN

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Introduction

A fully dynamic predictive model for assisting the implementation of the Precision Fish Farming approach in inland cultures of Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) is being built up under the H2020 Project NewTechAqua (New Technologies, Tools and Strategies for a Sustainable, Resilient and Innovative European Aquaculture, Grant Agreement 862658). At the core of this approach is the development and application of a bioenergetic mathematical model for simulating fish growth and metabolism. This bioenergetic model has been applied to real rearing cycles of both target species, delivering long-term predictions (months to years) of weight and metabolism indicators, based on input data characterizing the forcing functions – water temperature, and feed quality and quantity (Lima et al., 2022). Notably, the Root Mean Squared Relative Errors (RMSREs) between predicted and measured weights along the four monitored fish growth cycles were below 10%. Despite these robust long-term estimations, the water temperature alone cannot describe certain unusual environmental conditions which affect the mid-term (weeks to months) productivity of the system.

Here the bioenergetic model is expanded to include the effect of dissolved oxygen (DO). Specifically, the DO saturation level is introduced as a forcing, along with the water temperature and feed quantity and quality. Recent laboratory experiments (Araújo-Luna et al., 2018, McNicholl et al., 2021) have quantified the relationship between DO levels and feed intake and growth for Gilthead seabream. Hypoxia events which may sporadically occur in aquaculture ponds thus affect fish metabolism and the success of farmed fish operations. The proposed model is aimed at providing information for supporting the management of not only the DO supply, but also feed, and sheds light on the link between DO and feed intake.

Methods

The mathematical formulation of the bioenergetic model is based on that of Brigolin et al., 2014, in which the time derivative of the fish weight is described as the difference between the anabolic and catabolic rates divided by energy density of body tissues. Although DO levels also affect the catabolic rates, here the DO saturation is introduced as a component of a hyperbolic function which is directly linked to the feed intake included in the anabolic rate component of the model. The model is applied to three pilot growth cycles of seabream and one of seabass in 500–600m^3 ponds at the Il Vigneto farm, Tuscany, Italy. Water temperature and oxygen concentration data are obtained from probes manufactured by Endress+Hauser, deployed continuously in the ponds along several months and recording data typically once every hour. This monitoring system was designed and implemented in the NTA project for testing the model. Data for the remaining forcings, i.e., feed quantity and quality, are provided by the farmer.

Results and discussion

With the introduction of the effect of DO levels, the RMSREs between calculated and measured fish weights decreased for all the evaluated growth cycles. Figure 1 shows the evolution of the mean fish weight calculated by the state-of the art model (DO not included) and the new model (DO included), along with sampled weights, for one of the evaluated seabream cohorts. The bioenergetic model is run using daily data sets and equally returns daily values of fish weight. The only sampled weight input into the model is the initial weight. For the subsequent times, the model results are calculated independently from the sampled data. Figure 1 shows that the inclusion of DO, apart from increasing the overall accuracy of the model, particularly improves the description of the dynamics in periods when the fish growth is stalled, reducing the amplitudes between local peak-and-troughs and expressing weight growth trends closer to the measurements. Given that these periods of relative stagnation last for few months, information on trends of feed intake and fish growth in these critical times is certainly relevant for preventing overconsumption of resources.

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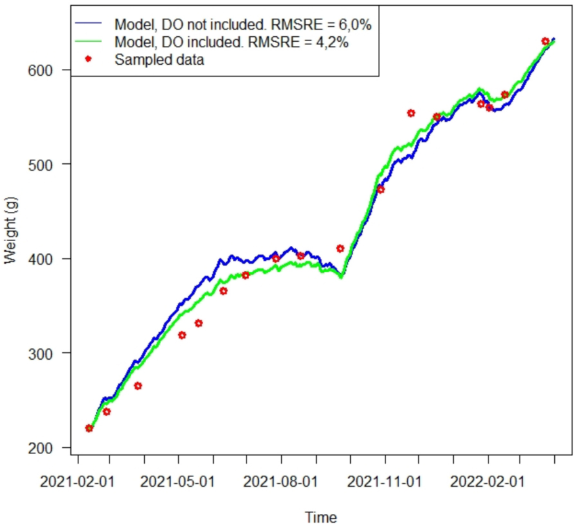

Figure 1. Individual fish weight evolution, selected case of gilthead seabream.

Acknowledgements

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APPLICATIONS OF HYPERSPECTRAL IMAGING FOR DOCUMENTING SMOLTIFICATION STATUS AND WELFARE IN ATLANTIC SALMON

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Introduction

Norway is the largest producer of Atlantic salmon (*Salmo salar*), with more than 300 million salmon put out to sea each year (Sommerset et al., 2020). In addition, in 2020 1,701,347 Atlantic salmon were reported used for research in Norway, which accounted for 74.5 percent of the total number of reported research animals (Kristiansen et al., 2021; Mattilsynet, 2021). In both research projects and commercial salmon farming, the welfare of the fish in a production unit is monitored by welfare indicators (WIs) that are either operational (OWIs) or laboratory-based (LABWIs). Morphological OWIs are for the most part manually assessed on a sub-sample of individual fish from the production unit. Examples of morphological OWIs are fin damage, eye damage, skin damage and gill damage, among others. Each OWI can be scored in different ways but an increasingly common approach is to score the injuries on a 0-3 scale, where 0 is basically undamaged and 3 can be classified as a severe injury (Noble et al., 2018). The process of manually assigning these welfare scores is time consuming and prone to both intra- and inter-observer error due to the subjective nature of the scoring method and the motivation and skill of the observer, amongst other factors. This paper discusses the feasibility of using hyperspectral imaging as a rapid and objective method of documenting and quantifying a selection of morphological OWIs.

Material and methods

We sampled n=1834 fish in total, across four experiments, and developed methods for image analysis based on machine learning algorithms using spectral data in the images. Automatic scores from the image analyses were compared to manual scores of WIs for each fish and this was used to evaluate the agreement between the two methods. In all experiments, hyperspectral images were taken on both the left and right side of each fish. The fish were presented to the camera on a conveyor belt moving at a speed between 10 and 20 cm/s. Morphological OWIs were recorded by trained manual observers for the same fish and used as reference data. For each WI the correlation between the hyperspectral index and the manual WI score was calculated. In trial 1, the dorsal fin was analyzed and compared with the manual OWI. The interrater agreement between 2 observers for dorsal injuries was calculated to be 0.66 in percentage agreement with a Spearman's correlation of 0.56 and a Cohen's kappa of 0.40. In trial 2, hyperspectral images of 120 fish, sampled before, during and after smoltification, were analyzed and correlated with smoltification status, assessed by quantifying plasma chloride levels after a 24-hour seawater challenge test. In trial 3, the use of hyperspectral imaging to evaluate eye haemorrhaging was tested. In trial 4, the potential of hyperspectral imaging to detect the degree of sea lice infection was assessed. The reference data in Trial 2 was a LABWI, namely blood serum chloride ion concentration after a 24-hour sea water challenge (Noble, et al., 2018) unlike the other trials which used OWIs as reference data.

Results and discussion

In trial 1 and 3 we observed the lowest correlation between the subjectively human scored OWIs and the hyperspectral scores (table 1). This can be due to a phenomenon called 'attenuation of error' where correlations are generally lower between two variables if one or both is measured with error (Adolph & Hardin, 2007). Part of the error can be attributed to the OWIs scored by human observers. Despite a standardized scoring scheme, different scorers weighted different aspects of an injury differently. Nevertheless, the correlation between the HSI scores and manual OWI scores in trial 1 is on par with the interrater agreement between two human scorers, which demonstrates that the hyperspectral system agrees almost as well with a human observer as two human observers agree with each other. The higher agreement for the LABWI supports this hypothesis. Counting lice is arguably less prone to subjective variation in scoring, although observer fatigue and thoroughness can influence the process, thus possibly resulting in a higher correlation in trial 4.

Conclusions

Together these results demonstrate the feasibility for using hyperspectral imaging as a technique for automatically monitoring welfare in Atlantic salmon and quantifying traits such as fin and eye injuries, smoltification status and lice infection levels.

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Table 1: Summary of the four trials and the agreement between manual welfare scores and the corresponding hyperspectral indexes.

	Number of fish scanned with HSI	Number of fish with manual reference	Indicator type	Feature	Agreement with manual WI
Trial 1	725	290	OWI	Dorsal fin injuries	0.54
Trial 2	849	120	LABWI	Plasma chloride	0.73
Trial 3	300	300	OWI	Eye injuries	0.55
Trial 4	1124	1124	OWI	Lice count	0.65

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METALS AND OTHER ELEMENTS IN FISH FARMS: *Posidonia oceanica* REVEALS THEIR DECADAL TRAJECTORY OF CHANGE

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Introduction

The development of fish farm activities in proximity to the Mediterranean coasts may lead to environmental impacts mainly related to the release of organic matter, nutrients, and different elements, like metals, and can have both direct and indirect effects on its marine macrophytes, including its endemic seagrass *Posidonia oceanica* (L.) Delile, (Boudouresque *et al*., 2020).

Seagrasses can serve as biological indicators due to the long-life span of their tissues, relative to the temporal scale of environmental variability, which allows them to integrate the biological signal of environmental quality though time.

In the present study, *P. oceanica* tissues were used as bioindicators of metals and element concentrations in proximity to fish farms units in the Aegean Sea, Greece. A dating technique was applied to reconstruct element concentrations in a decadal scale (2012-2021).

Material and Methods

During September 2021, we sampled *P. oceanic*a meadows in two sites in proximity to fish farm units located in the Aegean Sea, Greece (Chios and Oinouses Islands). In each unit, one station was chosen in proximity (40-80 m,) to the fish cages (hereafter called 'Cage') while another station was chosen as reference in safe distance (~ 800 m) from the cages (hereafter called 'Control'). In each station, fifteen shoots were randomly collected by divers in depths 3 to 5 m. Shoots from each station were dated using the reconstructive technique of lepidochronolgy (Pergent, 1990). All samples were lyophilized and homogenized, and element concentrations were measured in the sheaths, for each reconstructed year, using an Inductively Coupled Plasma Mass Spectometer (ICP-MS).

Results

The lepidochronological dating enabled us to reconstruct the period between 2012 and 2021 for each of our sampling stations. For each year, total concentration (μg g^{-1} dry wt) of thirteen elements (P, Mn, Fe, Co, Ni, Cu, As, Zn, Se, Sr, Mo, Cd, Pb) was measured in *P. oceanica* sheaths.

In both fish farm units, concentrations of the metals Cu, Zn and Mo were higher in "Cage" stations compared to the "Control" ones. In the fish farm unit of Chios Island, P, Mn and Cd concentrations were also higher in "Cage" (Table1). Linear regressions indicated that the concentrations in the cages of most of these elements (P, Mn, Cu, Zn) have shown no significant change within the investigated period (2012-2021). Cd was the only element that has increased by 0.05 μg g^{-1} dry wt y^{-1} ($P < 0.05$, R^2=49 %) in Chios Island, while Mo concentration in Oinouses Island has decreased by 3.52 μg g^{-1} dry wt y^{-1} ($P <0.001$, R2=78 %).

Discussion

Concentrations of P, Mn, Cu, Zn, Mo and Cd were found to be 1.3-fold to 2.2-fold higher in cages compared to the control stations. All of these elements have been previously associated to fish farm activities (Grigorakis and Rigos 2011, Kalantzi *et al*., 2021) Their elevated concentrations are most commonly related to fish feed ingredients and derive from uneaten fish pellets or feed excreta. Apart from the feeds, metal pollutants like Cu, often derive from antifouling paints used in the fish cages' nets (Ratcliff et al. 2016). Nonetheless, concentration of these elements has shown no significant changes within the last decade (2012-2021). Notice should be paid to the increase of Cd concentration in Chios fish farm, since it is a heavy metal, potentially toxic for the marine environment.

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Table 1: Mean ± SD element concentrations (μg g^{-1} dry wt) in *P. oceanica* sheaths during 2012-2021 and analysis of variance (ANOVA) between Cage and Control stations for the two fish farm units (Chios and Oinouses Islands). Only significant results are presented ($P < 0.05$).

		Mean (± SD)		**ANOVA**			
Chios	**Element**	**Cage**	**Control**	**df**	**MS**	**F**	***p*- value**
	P (%)	22.4. ± 11.8	13.1 ± 9.93	1	0.38	7.1	< 0.05
	Mn	25.46 ± 10.07	13.99 ± 7.67	1	657544860	8.2	< 0.05
	Cu	5.89 ± 2.89	3.00 ± 1.26	1	0.4	11.1	< 0.01
	Zn	24.24 ± 8.99	11.06 ± 6.23	1	868142731	14.5	< 0.01
	Mo	9.64 ± 4.16	5.40 ± 2.72	1	89855968	7	< 0.05
	Cd	0.69 ± 0.19	0.36 ± 0.16		533504	17.4	< 0.001
Oinouses	**Element**	**Cage**	**Control**	**df**	**MS**	**F**	***p*- value**
	Cu	7.77 ± 2.25	5.05 ± 1.18	1	37048799	11.4	< 0.01
	Zn	24.86 ± 5.62	19.19 ± 6.25	1	162418833	4.6	< 0.05
	Mo	15.70 ± 11.85	7.19 ± 4.11	1	363607351	4.6	< 0.05

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A NOVEL APPROACH FOR BROODSTOCK MANAGEMENT FOR OUT-OF-SEASON REPRODUCTION OF PIKEPERCH (*Sander lucioperca*)

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Introduction

Although quite extensively documented, out-of-season artificial reproduction of pikeperch still presents an issue of uncertainty in commercial hatcheries around Europe. Variability in outcomes is most often visible in fish individuality in response to hormonal stimulation presented in a wide range of latency times and egg quality over each broodstock (Żarski et al., 2019). Nevertheless, several thus far published papers pointed to stabilization of the procedure results with the experience of the breeders toward the reproductive cycle (Zakęś et al., 2013; Żarski et al., 2019; Ljubobratović et al., 2021). Thus, to get fully formed into a stable breeder, fish needs to undergo several reproductive cycles, which might be rather costly in terms of broodstock management economics raising the price of fingerling production. Therefore, we aimed to evaluate if the outdoor farmed breeders could be utilized for out-of-season reproduction batches following the shift of their natural reproductive cycle.

Materials and methods

In early November of 2021 and 2022, females were transferred from intensive outdoor conditions to fully controlled indoor RAS, while all the males were transferred in the first year and sperm quality parameters were evaluated in all of them in both years. During the five-week-long quarantine, the water temperature was raised from 10 to 18 °C and further transferred to a fully controlled climate room. Further on, fish were subjected to the simulation of summer, autumn, and winter as described by Ljubobratović et al. (2022). Finally, fish were artificially reproduced in the October of the following year as described by Żarski et al. (2019) and Ljubobratović et al. (2021), for SHIFT-1 and SHIFT-2. respectively. Egg quality was assessed in a total of 8 and 11 females in the first (SHIFT-1) and second (SHIFT-2) year, respectively.

Results

Female mortality in the period from transport indoors until the reproduction was 8.8 % in SHIFT-1 and 13.6 % in SHIFT-2, while the mortality of males in the first year was 2 % in the first year without any mortalities in the second year. All hormonally treated females ovulated and males spermiated in both years. Obtained embryo survival rate was 71.4 ± 10.8 % for SHIFT-1 and 63.2 ± 26.3 % for SHIFT-2 without significant differences between the two (Fig. 1). The progressive motility of the sperm of 20 evaluated males was 87.6 ± 8.3% and 83.2 ± 5.8 % for SHIFT-1 and SHIFT-2, respectively (Fig.1). A reduction in sperm motility was observed over the two consecutive years (*P* = 0.041, pair samples T-test).

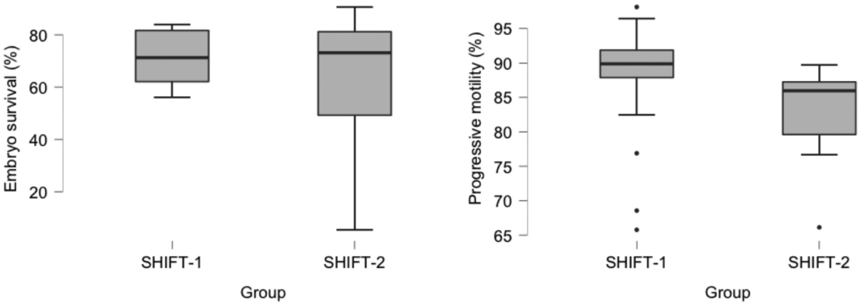

Fig.1. The embryo survival in 8 and 11 females following the reproductive cycle shift in first (SHIFT-1) and second (SHIFT2) reproduction trial as well as sperm motility in 20 males after first and second spawning occasion in out-of-season artificial reproduction of pikeperch *Sander lucioperca*

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Discussion

The presented outcome proves that mature pikeperch breeders are able to shift their cycle from natural to artificial and maintain a high quality of gametes. Noticed decrease in sperm quality in males should be the topic of interest in future studies. Likewise, the performance of females over the consecutive reproduction seasons should be evaluated. The procedure could be valorized for full-scale RAS farms in such terms to maintain one batch in partially controlled conditions suitable for preseason and seasonal reproduction while the breeders for out-of-season batches would be recruited following the evaluation of their reproductive performance.

Acknowledgements

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IFISHIENCI: GREATER AMBERJACK'S (*Seriola dumerili*) MICROBIOME MODULATION AS CONSEQUENCE OF CLIMATE CHANGE WARMING SIMULATION

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Introduction

Climate change impacts can dramatically affect marine food production systems. Marine microbial biodiversity has the potential to become a strong nature-based solution to increase the adaptation of the aquaculture industry to a warming planet. However, the aquaculture microbiome is still underexplored. In this work we aimed to understand the impact of water increasing temperature to both environmental and fish microbiomes in recirculating aquaculture systems and cascading effects upon fish growth.

Material and Methods

The thermal trial was conducted at certified laboratories (EL91-BIOexp-04; experimental reference Number, 255,344) where three temperatures (24ºC, 29ºC and 33ºC) were tested in triplicate tanks for three months. Bacterial diversity was monthly assessed from fish skin, gills and gut and tank water and sludge through 16S rRNA sequencing. Raw data was processed with QIIME2 and taxonomy analyses performed with R software.

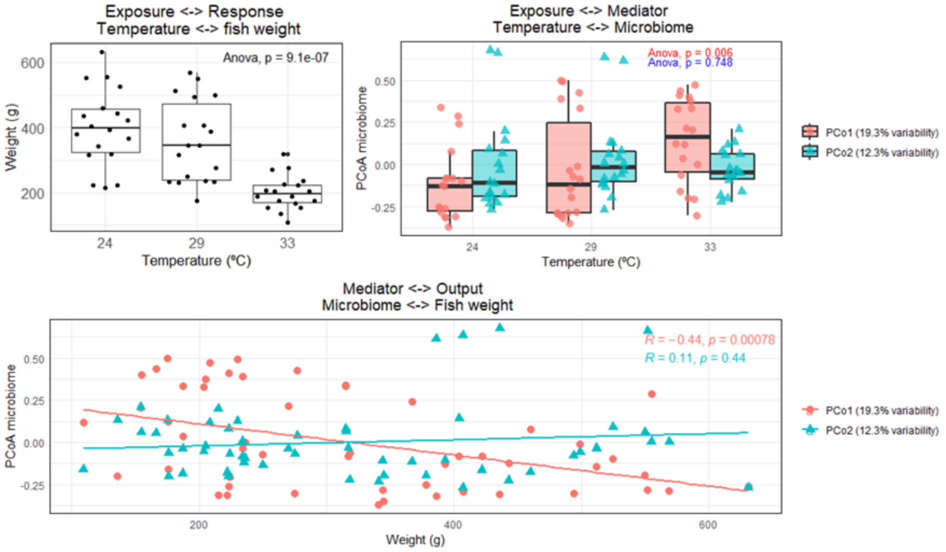

Fig 1. Association study between temperature treatment, fish growth and the microbiome. From left to right and top to bottom, we present the association between the weight and the temperature (top left); the association between the temperature and the microbiome community, summarized in the two principal coordinates of the Bray-Curtis PCoA (top right); and the correlation between the microbiome and the fish weight (bottom). For this analysis, we have considered the three months of experiment together, stratifying only by temperature treatment. The significance of each of the tested associations is presented in the corresponding panel, in the top-right corner. For the comparisons including the microbiome data, we coloured the corresponding points in correspondence tot the PCoA used.

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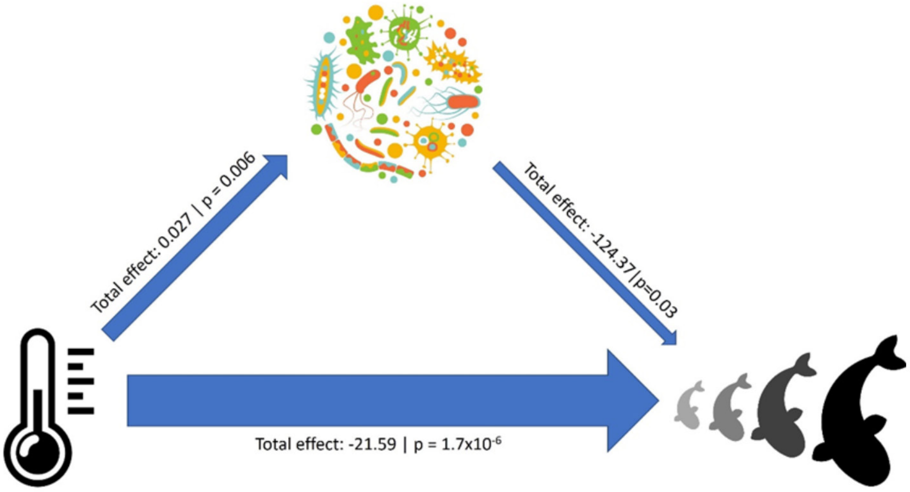

Fig 2. Mediation analysis summary results. The figure shows the effect of Temperature on fish growth and the microbiome and the microbiome effect on fish growth.

Results

Highest water temperature significantly impacted environmental and fish microbiomes and fish growth. Fish sections close to the exterior (gills, skin) were the most affected by temperature. Taxonomically, increase in temperature resulted in an increased abundance of thermo-resistant bacteria such as *Psychrobacter* and nitrogen-metabolism related ones, like *Acinetobacter*. Furthermore, genera such as *Enterovibrio* which has been described as having several pathogenic strains in fish, were only observed in this extreme temperature condition. Mediation analysis revealed a statistically significant influence of the differential microbiome composition upon fish growth.

Conclusions

Aquaculture climate change simulations result in fish microbiome alterations and compromised growth. Thus, paving the way to the development of microbiome-based tools to boost the adaptation of the aquaculture industry.

HISTOLOGICAL EVALUATION OF FLATHEAD GREY MULLET, *Mugil cephalus,* LARVAE REARED UNDER DIFFERENT CO-FEEDING STRATEGIES

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Introduction

Production of fish larvae is dependent on the use of live feeds for initial feeding. However, live food is costly, and may cause nutritional deficiency and low growth rates. A successful weaning strategy must ensure the production of well-fed larvae with fast growth and high survival, thus reducing production costs (Imentai et al., 2020). The histological study of the liver and intestine might support the evaluation of the nutritional status of larvae during the rearing process.

The flathead grey mullet *Mugil cephalus* is an emergent species for Mediterranean aquaculture diversification. However, little information on its larval nutritional requirements is available, and weaning still represents a bottleneck in this species (Loi et al., 2020). The aim of this work was to test three different co-feeding strategies on *M. cephalus* larvae, to find out whether reducing the level of Artemia sp. in the feeding protocol affected specific indicators of nutritional status, such as growth performances, hepatocyte vacuolization and intestine morphology.

Materials and methods

Larvae (22 dph), previously fed enriched rotifers, were divided into 3 experimental triplicated groups, at initial density of 4 larvae L^{-1} (1090 larvae in each 300 L tank). A 12h L: 12h D artificial photoperiod was set. Main water parameters were regularly monitored. Three weaning protocols: A100, A50 and A0 (2, 1, and 0 Artemia sp. ml^{-1} day^{-1}, respectively) were applied. For A100 (standard protocol), 5 enriched rotifers ml^{-1} day^{-1} were provided until 26 dph, together with 2 enriched Artemia nauplii ml^{-1} day^{-1} until 25 dph, gradually reducing the dose until 32 dph. In A50, the dose of Artemia was halved, while in A0 larvae received only rotifers until 32 dph (end of live prey period). In addition, a commercial microdiet (Gemma micro 0.1, Skretting) was offered in all treatments for the whole experiment, mixed 1:1 with Gemma wean 0.2 (Skretting) from 31 dph to the end of the experiment (36 dph).

Mullet growth performances were assessed at each of 3 sampling points, measuring total length (TL, mm), myotome height (MH, mm), Condition index (CI) and eye diameter (ED, mm). Five fish per tank were also preserved for histological analysis. Liver lipid area was assessed as described in Loi et al. (2020). At the end of the experiment, 10 intestinal fold height (Fh) and 10 enterocyte height (Eh) were measured in the histological sections of each fish and the mean values were used for the analysis. Finally, at the end of the experiment, mortality was also estimated.

Results

All the growth parameters increased over time. TL and MH were significantly ($p<0.05$) higher in the A100 treatment. CI and ED showed no statistical difference between A100 and A50, but in the A100 they were significantly higher than in A0 ($p<0.05$). The liver area covered by lipids grew significantly in the last experimental week, with no difference between treatments. In the intestine, Fh was significantly higher in fish fed Artemia, while Eh in A100 was statistically higher than in the other treatments. The lowest mortality was observed in A0 (35%), while A100 presented the highest mortality percentage (68%), followed by A50 (57%).

Discussion

ED resulted bigger in A100 larvae, implying a larger retinal image and an improved visual acuity (Papadakis et al., 2018). This suggests that fish were better able to catch preys than fish from other treatments and this may have led to a higher growth rate in the larvae fed standard Artemia dosage. The myotome development and the CI are sensitive markers for assessing inappropriate feeding conditions in fish larvae (Yúfera et al., 1993). The lower MH and CI measured in A0 may be a consequence of poor feeding conditions of the larvae fed rotifers only, as previously observed by Imentai et al. (2020).

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Histological observations of liver and intestine are considered indicative of the nutritional status of fish larvae (Przybyl et al., 2006). In this study, no significant effect of the dietary protocol was observed at the hepatic level; however, lipid coverage was slightly higher in fish fed Artemia and especially in those from the A100 treatment. This is possibly related to a higher amount of lipids in Artemia compared to rotifers (Imentai et al., 2020). The higher lipid coverage is consistent with better growth performances observed in the Artemia treatment. Significant differences were furthermore observed in the size of intestinal villi and in the height of the enterocytes, which were longer in fish from A100 than in fish from A0. Similar results were observed in pikeperch by Imentai et al. (2020), where larvae fed exclusively rotifers had significantly lower enterocyte height. It is thus plausible that fish from the A100 treatment were better-fed and grew faster as a consequence of a higher surface area for nutrients absorption.

In conclusion, fish fed standard dose of Artemia shown the best growth performances and hepatic and intestinal conditions. This may have been driven by the lowest final density observed in A100 due to the mortality rate, or by the highest nutritional value brought by the Artemia intake. Nonetheless, the data observed in the A50 treatment suggest that halving the Artemia dosage may still result in good larval performances and survival, and lower the production costs of live food.

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REARING *Mugil cephalus* JUVENILES IN FRESHWATER? YES, WE CAN!

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Introduction

Salinity potentially influences euryhaline fish growth, since the energy employed to maintain homeostasis is not available for development (Cardona, 2000). The optimal salinity for growth and metabolic rates depends on the species life history strategies and developmental stages (Morgan and Iwama, 1991). Exposure to less-than-optimal salinity, and the associated metabolic costs of osmoregulation, may influence the fish energy status and the lipids dynamics between the liver, the muscular mass and body fats reserves (Rabeh et al., 2015), which in turn may compromise growth performances.

Thanks to their low trophic status and their euryhalinity, mullets have recently become a species of interest for aquaculture. In particular, the flathead grey mullet *Mugil cephalus* is appreciated for its salted and dried egg roe, a traditional highly valued product. Despite its importance, little is currently known about its physiological performances under different salinity conditions. The aim of this study was therefore to compare the effects of two salinities on the growth performances, survival rates and nutritional status of juvenile *M. cephalus* born and raised under laboratory conditions.

Materials and methods

Five-month-old juveniles (N=380, 25.33±1.01mm length and 0.18±0.03g weight, mean±s.e.), from the same batch of eggs and reared in intensive conditions, were equally distributed into two experimental triplicated groups. Fishes were reared for 3 months at two salinity levels: freshwater (FW, 0ppt) and saltwater (SW, 36ppt). Fishes were fed *ad libitum* with artificial dry feed (Skretting).

At the end of the test, survival was assessed for each treatment. Growth was evaluated considering mean total length (TL, mm) and body weight (BW, g), Specific Growth Rate (SGR, % day^{-1}) and Condition Index (CI). Liver lipid coverage (ACLV) was histologically monitored as described in Loi et al. (2020). Total lipids, fatty acids, and other lipid bioactive molecules such as endocannabinoids (ECs) and N-acylethanolamines (NAEs) were assessed on juveniles' homogenized whole body.

Results

Survival rate was high and similar ($p>0.05$) between treatments. TL and BW grew over time, and juveniles reared in SW showed a significantly ($p<0.05$) higher SGR for BW. CI was slightly (not significantly) higher in fish reared in FW. ACLV increased along the experiment, with no difference between treatments. At the end of the trial, FW and SW did not differ in terms of total lipids and fatty acids content, while lipid signalling molecules NAEs (specifically AEA, OEA and DHEA) were found significantly higher in fish reared in FW.

Discussion

Either fresh- and saltwater resulted suitable for juvenile survival. The ability to tolerate freshwater is directly proportional to fish size, as demonstrated by Rodriguez et al., 1993 when recorded the lowest (<20%) survival of *M. cephalus* juveniles in freshwater. Interestingly, their experiment involved larger and older individuals compared to those used in the present study, which should have therefore better coped with low salinities. Our survival results similar to that observed for juveniles of another mullet species, *Mugil liza*, reared under salinities below 24ppt, which evidently do not negatively impact survival in this species (Lisboa et al., 2015).

Juveniles reared under the two salinities showed similar growth, except SGR for body weight, higher in SW. CI was slightly higher in juveniles reared in FW, suggesting that extending the experimental period could result in a better growth than at higher salinity levels. Previous works on the same topic are contradictory, suggesting better growth in saltwater (Lisboa et al., 2015), sometimes in freshwater (Cardona, 2000) or even at intermediate salinities (De Silva and Perera, 1976). This variability is possibly due to the different developmental stages of the fish used in the experiments, which can modify the energy cost associated with the maintenance of homeostasis (Lisboa et al., 2015).

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ACLV, total lipids and fatty acids resulted similar between treatments. On the other hand, the bioactive fatty acid derivatives NAEs were higher in fish reared in FW, suggesting a different activity of the FAAH2, the NAEs' degrading enzyme involved in hyperosmotic stress responses in other vertebrates.

In conclusion, fish reared in freshwater have similar performances to those reared in saltwater. Our data suggest that the transfer of mullet juveniles from saltwater hatchery conditions to freshwater is a viable commercial scale production strategy, and this could increase the number of suitable spaces available for *M. cephalus* aquaculture.

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SPONGE MARICULTURE IN THE REMEDIA LIFE INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM (SOUTHERN ITALY, NORTHERN IONIAN SEA)

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Introduction

The polyculture of fish with other organisms at different levels of the trophic web (Integrated MultiTrophic Aquaculture, IMTA) is an eco-friendly alternative that has received increasing attention (Bierwirth, et al 2022) due to its environmental benefit and promises considerable economic potential. Among the co-cultured organisms Porifera, or sponges, seem the most promising due to their high efficiency in removing organic particles between 0.1–50 μm (Simpson, 1984; Larsen and Riisgård, 1994; Riisgård and Larsen, 1995; Hadas, et al. 2006), capable to positively affect the quality of the surrounding water and their intrinsic economic value. The present study was undertaken to assess the survival, the growth rate and the effect of rearing methods in different sponge species within the Remedia Life IMTA system.

Materials and methods

The Remedia Life IMTA system, ongoing in the "Maricoltura del Mar Grande" aquaculture plant (Sothern Italy, Northern Ionian Sea; www.remedialife.eu), consists of fish cages producing *Dicentrarchus labrax* (Linnaeus, 1758) and *Sparus aurata* (Linnaeus, 1758), to which an innovative set of bioremediator organisms such as sponges, polychaetes, bivalves and macroalgae have been associated (Giangrande, et al. 2020). Among sponges five species were reared in: *Aplysina aerophoba* (Nardo, 1833), *Geodia cydonium* (Linnaeus, 1767), *Hymeniacidon perlevis* (Montagu, 1814) *Ircinia variabilis* (Schmidt, 1862) and *Sarcotragus spinosulus* (Schmidt, 1862). Specimens were cut into explants of similar size and arranged in the Remedia Life long-line system down to ~6 meters depth. Explants monthly monitoring was performed to evaluate the rearing performance in terms of survival and growth rate. For *S. spinosulus*, an additional comparison among three rearing techniques (fishnet sock, rope and net bag) by means of survival rate was carried out.

Survival rates were assessed by visual observation and calculated from the initial and final number (N) of explants as follows: Survival (%) = $(N_{final} / N_{initial}) \times 100$. Monthly growth rates were calculated measuring volume (V, mL) by water displacement as follows: Growth (%) = $((V_{final} - V_{initial}) / V_{initial}) \times 100$.

Results

All sponge species during the first year of rearing showed a high survival ratio, with values not lower than 82% (for *S. spinosulus*). Similarly, all species showed values of more than 6% monthly biomass increase (with values up to 16% for *A. aerophoba*), with the exception of *H. perlevis*, which showed a volumetric reduction of almost 2%. However, when statistically analysed (Mann-Kendall trend test, Gilbert 1987), this decrease was not significant (p (no trend) = 0.375), nor the increase of *G. cydonium* (p = 0.113); for the rest of the species, a significant increasing trend was detected (p values < 0.01).

Regarding the first-year culture methods of *S. spinosulus*, all of them resulted in a survival rate of more than 82%, reaching 91% of the cases when the net bag was used (figure 1).

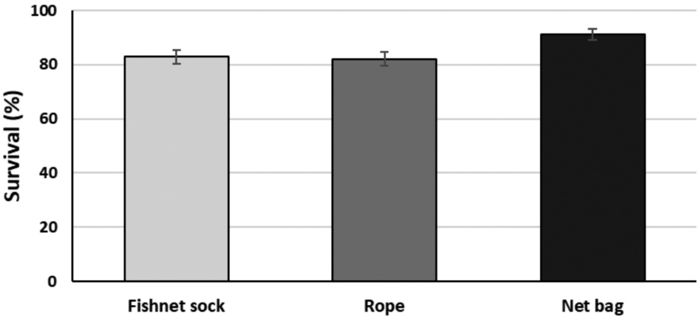

Fig. 1. Effect of the rearing method used in the survival (± SE) of *S. spinosulus*.

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Discussion and conclusions

Overall, all tested sponge species promise great results in the IMTA activity, with high survival and growth rates. When compared with same taxonomic subclass sponges reared in net systems, the five species cultured in Remedia Life IMTA plant show a higher mean survival rate, even when rope system was used for *S. spinosulus* (Bierwirth et al. 2022). Growth, however, is a less studied parameter among *in situ* cultured sponges of the selected species and makes the comparisons complicated, which at the same time highlights the importance of the present research in order to improve productivity in this type of aquaculture systems. All in all, the increase up to 300% of additional biomass (for *S. spinosulus*) and high growth rates (as for *A. aerophoba*) together with their demonstrated filtering activities (e.g. Wehrl et al. 2007; Trani et al. 2021) make these sponge species ideal candidates for co-culturing in IMTA plants.

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ACUISOST: AQUACULTURE SUSTAINABILITY AND RESILIENCE THROUGH NUTRITIONAL STRATEGIES

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ACUISOST is a project funded by Next Generation EU funds and born inside of ACUIPLUS Cluster. The main goal of ACUISOST is to include the concept of sustainability in aquaculture as a cornerstone of the Blue Economy, as stated by the European Union (Strategic Guidelines for the Sustainable EU Aquaculture), as well as the Spanish authorities (UN Sustainable Development Goals). By this means, ACUISOST aims to explore the use of new nutritional strategies, together with technological additives, that could lead to the manufacturing of new, functional and sustainable fish feeds. This innovation is intended to improve resilience in fish industry, by enhancing fish production, health and welfare under the aquaculture production system and overcoming climatic change issues; thus, promoting a more resilient and sustainable EU aquaculture industry.

As that is an ambitious goal, ACUISOST is not alone in this huge challenge and counts with the backup of many research institutes (and universities), as well as cutting edge companies. The partner companies of this project are: DIBAQ S.A, Health Tech BioActives (HTBA), NATAC Biotech, TEBIO and FAES FARMA – TECNOVIT.
Together, we are committed to develop the following activities:

- Development of new fish feeds based on insect meal along the production cycle as a sustainable substitute of fish meal.
- Development and validation of new sustainable functional fish feeds as an alternative strategy to anti-microbiological compounds, by including bioactive plant-based compounds (phytogenics).
- Inclusion of sustainable additives on aquafeeds to improve the nutritional quality and shelflife of the fillet at chilling and freezing temperatures.

These studies will take place in an integrated and novel way (preliminary screening and functional evaluation of additives of interest by *in vitro* approaches using different cell cultures, followed by their validation by means of an *in vivo* trial), as well as under a scenario focused on climate change. These studies will be addressed in two of the main European farmed species, the gilthead seabream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*). In addition to this, communication and dissemination plans have been designed to share the knowledge and innovation generated by the project to stakeholders and the general public in order to maximize the socio-economic impact of the project.

ACUISOST has created a multidisciplinary and integrative consortium in which research institutions (IRTA, University of Barcelona, University of Vigo, University of Murcia and ICTAN, CSIC) collaborate together with industrial stakeholders in order to achieve the project's goals. Consequently, the proposal will create a multidisciplinary, inclusive, and cross-cutting framework where the skills and competencies of each of the partners will allow the successful achievement of the project's objectives and generate a positive impact on the aquaculture industry and therefore, on society. In this context, the project is the result of exhaustive study work, in which various scientific groups with a multidisciplinary profile and diverse areas of specialization are involved, but which are rooted in a common point: the sustainable development of aquaculture, under principles of animal welfare, nutrition and circular economy, in which cooperation between the key players, and the transfer between the field of research and industry is key to this development.

During the event, ACUISOST strategy will be described in more detail and the involvement with other key players in the aquaculture industry will be discussed.

Acknowledgements

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ANALYSIS OF DIFFERENTIATION AND INNOVATION STRATEGIES FOR FISHERY AND AQUACULTURE PRODUCTS IN FRANCE AND SPAIN

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Today, salmon aquaculture is considered the fastest growing food production system in the world, accounting for 70% (or 2.4 million metric tonnes) of the market (Shepherd & Little 2014). However, since the mid-1990s, the real price has remained relatively stable, indicating that demand growth has kept pace with productivity growth (Asche, F., et al., 2011).

This development has also led to the creation of high-value cuts such as loins, similar to chicken fillets, as well as some products based on nuggets, trimmings and other cuts. Developments in this regard have been limited and whole fish and fillets, whether fresh, frozen or smoked, remain the main end products (Asche, et al., 2018).Study of the different products derived from the Salmoneidae family found in a common supermarket (Carrefour) in two different countries (France and Spain). The objectives of this study are described below.

We constructed a database from products derived from salmon and marketed by the websites of Carrefour France and Carrefour Spain. In Carrefour France, the most present group is still smoked salmon fillets (38%), however, in France the quantity of cooked by-products (36%) is much higher than in Spain (14%) and the next most abundant group is preserved by-products (9%). But for Carrefour Spain which belong to the groups established in the previous sections. It can be seen that most of the products marketed in this chain in Spain belong to the group of smoked salmon fillets (35%), the vast majority of which are marketed as thin slices of smoked salmon. Next, the groups with the greatest number of options are frozen loins (21%) and cooked by-products (14%). As for the other formats, they are represented in very small quantities.

The products with the highest price increases are those with sustainability certifications. This is followed by the products that do not present any kind of specification, closely followed by those with health claims. The difference between prices as a function of differentiating characteristics is significantly different (at all levels according to Tukey's test), with a p-value of 0.32.

The products with the highest value change depending on the country, for example in France, those of superior quality are those with the highest price, whereas in Spain the most highly valued products are those with some alteration in taste, and in this group there is hardly any difference between Spain and France. The difference between prices as a function of innovations is significantly different.

Freshness is one of the most important quality criteria for buyers of Atlantic salmon (Badiola, et al., 2017) and fish products in general and this is perhaps why traditional formats (fillet cuts with preserved shapes that guarantee the highest freshness to the consumer (Asche, et al., 2018)) are dominant in supermarkets.

EUROPEAN CONSUMERS' SUSTAINABILITY BELIEFS: WILD FISH *VERSUS* FARMED FISH

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Introduction

Aquaculture may play a crucial role in global sustainability as a valuable source of food and preservation of fish stocks by reducing wild fish catching. Even so, consumers still have a worst perception of fish from aquaculture when compared with their wild counterparts. This worst perception is sometimes translated into lower consumption, as in the European Union (EU) most of the fish consumed comes from wild fisheries (75%) (European Commission, 2020), a higher proportion than the global average (48%) (FAO, 2020). Therefore, understanding European consumers' beliefs about farmed and wild fish is deemed key in building an effective strategy to communicate aquaculture sustainability to consumers.

The main objectives of this study were 1) to assess European consumer sustainability beliefs when farmed and wild fish are compared, and 2) to identify segments of consumers with similar beliefs about fish sustainability.

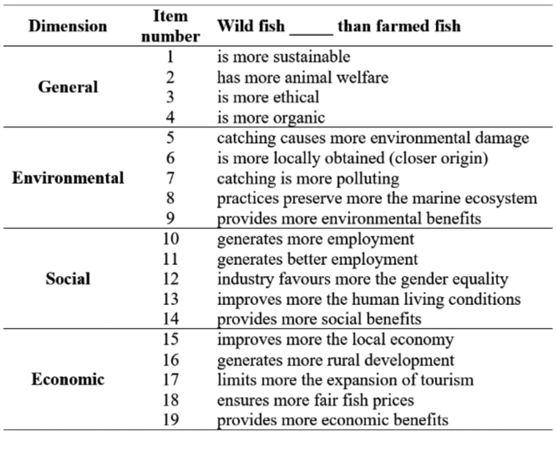
Materials and methods

An online survey was answered by 2,145 fish consumers from five EU countries (France, Germany, Italy, Poland, and Spain). The survey assessed 19 participants' beliefs comparing the sustainability of wild and farmed fish (Table 1). Beliefs covered four areas, including general aspects of sustainability and its three dimensions (environmental, social, and economic). All beliefs were presented in the format "Wild/farmed fish _____ than farmed/wild fish". A 7-point Likert scale, from 1 (strongly disagree) to 7 (strongly agree), was used to measure each belief.

Statistical differences between participants' beliefs from different countries were explored through an ANOVA with Tukey's test.

A two-step cluster analysis was performed to identify segments of consumers with similar beliefs followed by an ANOVA with Tukey's test to find statistical differences between segments. Finally, those segments were profiled using various sociodemographic variables, including country, age, gender, education level, perceived economic situation, and place of living (rural-urban and inland-coastal).

Table 1. Selected beliefs comparing wild *versus* farmed fish.



Dimension	Item number	Wild fish _____ than farmed fish
General	1	is more sustainable
	2	has more animal welfare
	3	is more ethical
	4	is more organic
Environmental	5	catching causes more environmental damage
	6	is more locally obtained (closer origin)
	7	catching is more polluting
	8	practices preserve more the marine ecosystem
	9	provides more environmental benefits
Social	10	generates more employment
	11	generates better employment
	12	industry favours more the gender equality
	13	improves more the human living conditions
	14	provides more social benefits
Economic	15	improves more the local economy
	16	generates more rural development
	17	limits more the expansion of tourism
	18	ensures more fair fish prices
	19	provides more economic benefits

(Continued on next page)

Results

When both production systems were compared, European consumers perceived better the welfare of wild fish, also being more organic, but causing more environmental damage. Farmed fish, instead, was perceived as being more sustainable, being more locally obtained, providing more environmental benefits, generating more employment, improving the human living conditions, generating more rural development, and ensuring fair fish prices.

Four segments of consumers with similar sustainability beliefs were identified. The "pro wild fish" segment (n=653 respondents) grouped participants whose beliefs favoured wild fish. They held the belief that wild fish welfare was better, was more organic, was more ethical, caused less pollution, preserved the marine ecosystem, and improved the local economy. This segment included more females and more French respondents, but less Polish.

The "pro aquaculture" segment (n=472) grouped participants whose beliefs favoured farmed fish, although two exceptions were found. Respondents perceived better welfare of wild fish and also being more organic. This segment grouped older participants, more males, and more Italian and Spanish respondents.

The "strong convictions" segment (n=420) grouped participants whose beliefs sometimes were clearly in favour of wild fish, whereas others were clearly in favour of farmed fish. This segment grouped younger participants, with a higher perceived economic situation, living in urban and coastal areas, from Germany, Poland, and Spain.

The "impartial" segment (n=600) grouped participants whose beliefs did not follow any trend favouring or refusing the sustainability of one or another production system. More French and Polish participants were included in this segment.

Conclusions

The role that aquaculture may play in global sustainability is crucial. Such importance should be translated to citizens with the final aim of increasing farmed fish consumption. Therefore, understanding how European consumers perceive sustainability of farmed fish when compared with wild fish is the first step in improving consumers' perception of aquaculture. The identification of four segments of consumers and its profile could be useful to conduct tailored promotional campaigns to communicate aquaculture benefits and debunk its myths.

Acknowledgments

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LOOKING FOR THE BEST WAY TO INCREASE CONSUMERS' AWARENESS ABOUT AQUACULTURE

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Introduction

No matter how important a message is if it is not able to reach the audience. In this sense, despite the multiple advantages of the aquaculture production and the role that it can play in global sustainability, these messages often do not reach the general population due to the existence of a gap between experts' knowledge and consumers. Therefore, finding new channels and ways to reach consumers is crucial to assure the full development of the aquaculture sector and to promote farmed fish consumption.

This study aimed to involve aquaculture sector stakeholders to generate new strategies to increase consumers' awareness of European aquaculture.

Materials and methods

Two creative sessions were carried out in each of the three selected countries (i.e., France, Italy, and Spain), one with four aquaculture experts and the other with eight fish consumers. Experts met the criteria of having a high knowledge and experience about the aquaculture sector (e.g., researchers, professors, farm managers, etc.). Conversely, consumers met the criteria of being around 50% female and 50% male, older than 18 years, responsible for food purchase and preparation within their household, and fish consumers.

Various creative techniques were used to stimulate participants' creativity, including brainwriting, direct analogies, and empathic thinking. The multiple ideas generated in the creative sessions were grouped through an *ex post* process to give structure to the data.

Results

The creative techniques used during creative sessions allowed participants to generate a pool of strategies to increase the awareness of the consumers about European aquaculture. Among the ideas arisen, some were related to the **fish selling places**, such as supermarkets. In this sense, doing tasting or show cooking *in situ* may bring the fish product closer to consumers and, consequently, increase their awareness. Also, giving them brochures or free samples for taking home and share it with the family. Finally, the role that can play personnel's recommendation should not be neglected.

Restaurants are also frequent fish selling places in which consumers can taste different aquaculture species and to test them against their wild counterparts. Even though, as preconceived ideas may play a negative role against farmed fish, a participant proposed to inform the clients, once they have finished eating their dishes, that the fish they just have eaten comes from aquaculture. In addition, a "tapas" route only using aquaculture fish may also encourage participants to taste the product as well as a cooking class in which participants can cook their own fish and eat it later.

If the focus is on **fish packaging**, participants come out with various ideas related to including pictures on the label such as a logo, a quality scheme as PDO (Protected Designation of Origin) or PGI (Protected Geographical Indication), a picture/information about aquaculture, or a QR code that shows informative content. Finally, one participant proposed to print on the label information about a prize, in which the prize is a trip to aquaculture facilities with a tasting.

Many ideas came out related to doing **activities** centred around aquaculture, some examples are to do outdoor events to spend the whole day with the family, to organise the Aquaculture Week plenty of events (e.g., Fashion Week), to celebrate the World Day of Aquaculture, or to create an amusement park about aquaculture.

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Some of the activities proposed were in fish **farm facilities**, including organising visits, trips, and guided tours, also for scholars. Organise activities and experiences in seaside or lakes during Summer, a Summer camp for teenagers, or a spot for vacations (e.g., stopping point for motorhomes). Experience a different vacation with a working exchange: work in exchange for housing and food. Doing tastings (e.g., wine cellars), even offering two activities: fishing and eating the fish they just have fished. Organise a competition on who fish more fishes in 5 minutes. Focusing on children, creating an educational path with cartoons or animated videos, or touching and cuddling marine creatures.

Advertising also came out during the sessions. Various channels were mentioned, including television, social media, supermarkets and other selling places, local and ethical markets, posters, signs, brochures, sponsor of show cooking, aquariums, etc.

Education was considered of primordial importance. For example, dedicating more hours at the school to aquaculture or doing experiments at Science subject (e.g., design a Biofloc system). In addition, giving lectures at universities or organising workshops. Also, to learn while playing, creating **board games** for children or **home kits to play** with (e.g., build an "aquaponics" system at home).

Regular communication channels also were stated by participants. For example, casting on **television** documentaries, entertainment programs, debates, interviews, short movies, series, cartoons, famous people talking about aquaculture, programs like "big brother" but for fishes. **Newspaper and magazines** also were mentioned, as well as informative programs or songs for children on the **radio**. More recent communications channels also came out, including **podcasts on Spotify** and **videos on YouTube**. Participants give various examples of the type of content that may be published on YouTube, including food blog channels with receipts using farmed fish, competitions or prizes, and child-accessible protected channels. Finally, **social media** also were mentioned.

Surprisingly, social media did not come out as many times as an expected. The main reason may be participants' age, as younger generations tend to use more frequently social media, although there were few participants between 18-30 years. Therefore, it would be worthwhile to inquire how to communicate to children and teenagers through creative sessions involving only youths. As pointed out by various participants of this study, educate younger generations is essential as they are the future aquaculture fish consumers.

In addition, some participants come out with an invaluable concept as is the "multichannel" or "omnichannel" strategy. That is, approaching consumers not only through one channel but with multiple ones. In a similar vein, an Italian consumer stated, "The promotion of aquaculture must be "multi-level" (institutions, schools, and media) and "multi-disciplinary" (science, market, economy, and health)".

Conclusions

Creative sessions were useful to generate a pool of new strategies to increase consumers' awareness of European aquaculture. Producers and marketers could benefit from the ideas generated to choose the best channel or channels to reach consumers with the final aim of designing more effective aquaculture communication and promotional campaigns.

Acknowledgments

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AQUACULTURE OF RED KING CRAB *(Paralithodes camtchaticus)*: EFFECTS ON MUSCLE PROPERTIES

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Introduction

Red king crab (RKC, *Paralithodes camtschaticus*) has become important for the shellfish industries located in the northern parts of Norway. In fact, in 2020 and 2021, 2016 and 2261 metric tons of RKC was exported from Norway, amounting to NOK 667 and 999 million, respectively. About 60% of the exported volume was traded live, whereas the remaining share was exported as processed fresh or frozen-cooked clusters.

An alternative to the live export or processing right after landing is aquaculture. Aquaculture, with or without feeding enables the industry with a more flexible management of the resource. Besides, aquaculture also facilitates for recovery and improved welfare in case of a rough handling during the capture.

The present study was undertaken to study how the muscle of processed RKC was affected by different aquaculture conditions.

Material and methods

After capture nearby the North Cape in northern Norway, the RKC was transported, landed, and kept in tanks. To screen potential effect from the aquaculture conditions, parameters like water temperature, access to feed, duration, and the time of the year, varied. During the period for aquaculture, the RKC was sampled, slaughtered, processed, and the quality of the muscle was evaluated. The evaluation included meat content, yield, water content, pH, water holding capacity, and microbial growth.

Conclusion

The conditions for aquaculture of RKC clearly affects the properties of the processed muscle. In the future, detailed knowledge on how the aquaculture conditions influences the final quality of the RKC muscle is considered to be of crucial importance.

THE EFFECT OF FEEDING WITH THE COPEPOD *Acartia tonsa* DURING THE FIST DAYS OF LARVAL REARING ON SKELETON ONTOGENY AND SKELETAL DEFORMITIES IN GREATER AMBERJACK (*Seriola dumerili*)

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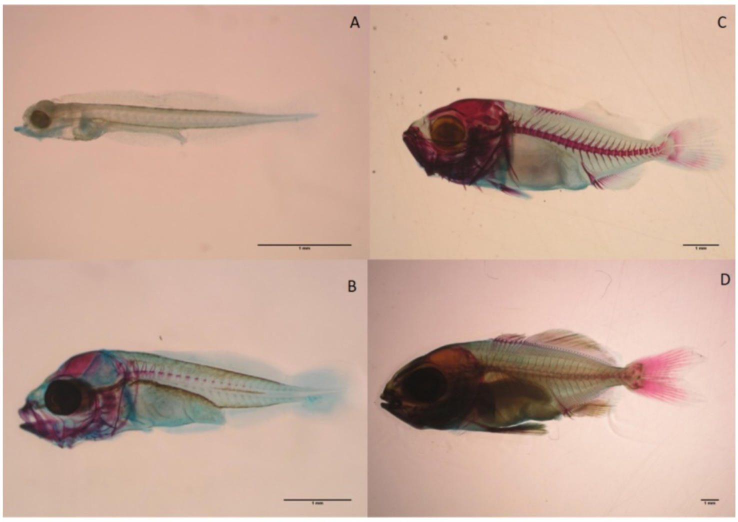
Introduction

Ontogenesis is a process of growth (change in size with age) and differentiation and maturation (cells, tissues, organs and systems) (Chambers and Leggett, 1987), where fish larvae undergo dramatic changes in body shape, in their swimming ability, metabolism and behavior. Nutrition is one of the most important factors affecting the ontogeny of fish which among other things affects skeletal ontogeny and therefore may be responsible for the development of skeletal abnormalities (Chambers and Leggett 1987). Copepods have the advantage that the fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are found in the phospholipids of cell membranes. Copepods do not need enrichment like rotifers and *Artemia* metanauplii and so do not burden the surface of the tank with extra fat (Conceição et al. 2010). In addition, copepods are very rich in free amino acids which stimulate the sense of smell of fish larvae and enhance feeding behavior and food search in the tank (Rønnestad et al. 2013).

Materials & Methods

In a commercial fish farm, four cylindroconical tanks were stocked with 3,000 L in each, and we started feeding the larvae 3 days post hatching (DPH) until 30 DPH. In two tanks, the fish larvae were fed with *Acartia tonsa* and *Brachionus* sp., while in the other two only with control animals. On the eleventh day post hatching, fish larvae of all the tanks, started feeding on Artemia nauplii as well. About day 19 all the fish larvae were fed with rotifers, *Artemia* nauplii and large enriched *Artemia* metanauplii. In the last days of breeding, formulated diet was also added. Samples were taken every three days and fixed in 5% formalin (pH = 7.2). Bones and cartilages were stained with Alizarin red and Alcian blue respectively (Park & Kim 1984).



Pic 1: Different stages of development in larvae and juveniles of *S. dumerili*, stained with red and blue for bones and cartilages respectively (A: 3.3 mm, B: 5.1 mm, C: 8.8 mm, D: 18.1 mm) (Scale 1 mm).

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Results

In picture 1(a-d) you can see the different stages of development in larvae and juveniles of *Seriola dumerili*.

The majority of the structures appeared earlier in the fish larvae which were also fed with copepods. Exceptions are the Proximal Pterygiofores (**Prx**) and the Distal Radials (**Rd**) of the dorsal fin and the Upper and Lower Supratemporal (**SutUp-Lo**) of the pectoral fins that appeared earlier in the fish larvae which were fed only with rotifers. The osteologic development was completed earlier in the fish larvae which were also fed with copepods, excluding the **Rd** in the dorsal and anal fins, the **Prx** in the pectoral fins and the Lepidotrichia (**R**) in the pelvic fins. The ossification was completed earlier in the copepod-fed larvae. More skeletal deformities were observed in the control group with the exception of the dorsal and the pectoral fins. In the pelvic fins no deformities were observed in either groups.

Conclusions

In conclusion the use of copepods in the breeding of *Seriola dumerili* affected positively the beginning and the completion of the osteological development, the completion of the ossification and the number of skeletal deformities.

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BACTERIA ASSOCIATED WITH THREE BIVALVE SPECIES OF COMMERCIAL CONCERN – THE SEEBug PROJECT

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Introduction

Bivalve molluscs accounted for 18 million tonnes of aquaculture production in 2018, what represented an increase of *ca*. 3 million tonnes compared to the past years (2010-2015) (Wijsman et al., 2019; FAO, 2020). Despite the efforts to produce and commercialize safe and high-quality seafood, once these organisms are filter-feeders, the accumulation of chemicals and biological contaminants are a huge concern to public health (Oliveira et al., 2013; Sousa & Hinzmann, 2020). This is particularly worrisome since bivalves are commonly consumed live, raw, or slightly cooked (Oliveira et al., 2013; Anacleto et al., 2014; Lattos et al., 2021). Hence, the present study aims to I) to detect and identify the diversity of cultivable pathogenic bacteria isolated from three commercially valuable bivalve species, before and after being depurated and II) evaluate the efficiency of the depuration process in reducing cultivable bacterial load. This work has been developed under the project SEEBug (Development of a sensor for the fast and efficient detection of pathogenic bacteria in Bivalves) funded by MAR2020, and it intends to identify other relevant bacterial pathogens that should eventually be considered within bivalves microbiological monitoring programs.

Materials and methods

This study focused on the bivalves *Venerupis corrugata*, *Cerastoderma edule* and *Solen marginatus* in two debug states. They were harvested in Ria de Aveiro during two seasons of 2021 (summer and autumn). For the purpose to detect and isolate pathogenic bacteria, two procedures were applied on non-depurated (ND) and depurated (D) bivalves, based on: (i) International Organization for Standardization (ISO) 16649-3, ISO 6887-1, and ISO 6887-3 standards for enumeration of *Escherichia coli*, and (ii) the use of selective (MacConkey (MC) and Thiosulfate-Citrate-Bile Salts-Sucrose agar (TCBS)) culture media to quantify and isolate the cultivable bacteria colonizing bivalves. Procedure I allowed determining the MPN (Most Probable Number) of *E. coli* per 100g of bivalve. In Procedure II was analyzed the number of Colony Forming Units (CFU) per 100g of bivalve. For the latter Procedure a Student's *t*-test was performed to identify statistically significant differences in the bacterial CFU detected in each debug state (ND *vs*. D) of the bivalves species under study. Posteriorly, the isolated bacterial clones were subjected to molecular identification through sanger sequencing of the 16S rRNA gene.

Results and discussion

Procedure I data (Table 1) showed that the MPN of *E. coli* in non-depurated *C. edule* in the summer season was below the limit established in current legislation (EU, 2019). On the contrary, the other bivalves species revealed higher values. In autumn, the *E. coli* MPN values in *V. corrugata* and *C. edule* bivalves were within the legally allowed range for human consumption. As expected, in both seasons was observed that after the depuration process there was a significant decrease of *E. coli* MPN/100g bivalve, thereby apparently revealing the efficiency of the depuration method for *E. coli*. However, this trend was an exception for *V. corrugata*, which *E. coli* levels in summer remained equal before and after depuration. Regarding Procedure II, it was observed a decrease of CFUs after the depuration process, except for *S. marginatus* (Fig. 1 (a)) and *C. edule* (Fig. 1 (b)), where it was verified a increase of CFUs. Furthermore, 18 genera of cultivable bacteria were isolated, and some of them represent danger to public health given their pathogenicity. In summary, even though the depuration process appears to be efficient to reduce *E. coli* it may fail to reduce/eliminate other potentially harmful pathogens as *Pseudomonas*, *Serratia* and *Vibrio*.

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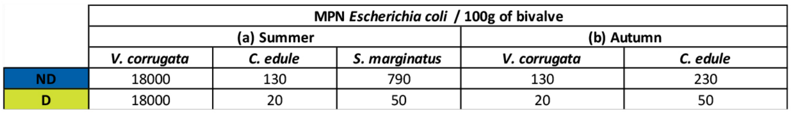
Table 1 - Mean MPN of *E. coli* obtained in Procedure I for the non-depurated (ND) and depurated (D) *Venerupis corrugata*, *Cerastoderma edule* and *Solen marginatus* samples harvested in **(a)** summer and **(b)** autumn.



	MPN *Escherichia coli* / 100g of bivalve				
	(a) Summer			(b) Autumn	
	V. corrugata	*C. edule*	*S. marginatus*	*V. corrugata*	*C. edule*
ND	18000	130	790	130	230
D	18000	20	50	20	50

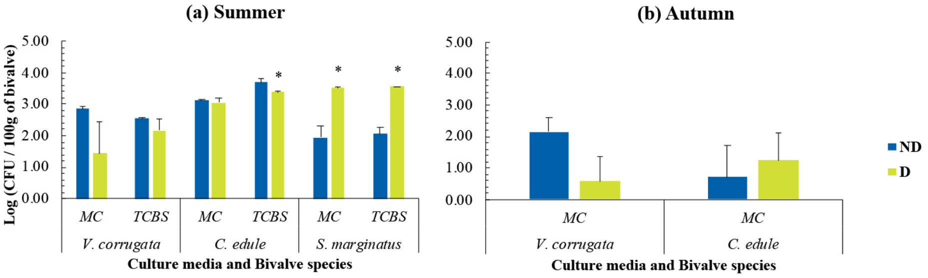


Fig. 1 – Colony Forming Units (CFU/100g bivalve) determined by procedure II in **(a)** summer for *Venerupis corrugata* (N = 45), *Cerastoderma edule* (N = 45) and *Solen marginatus* (N = 14) (harvested in July) in each culture media (MacConkey (MC) and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar); and **(b)** autumn for *V. corrugata* (N = 45) and *C. edule* (N = 45) bivalves (harvested in September) in MC medium. No growth was observed on TCBS agar in autumn. Error bars represent standard deviation. * represent statistically significant differences ($p \leq 0.05$) between debug states.

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ASSESSING THE EFFECTS OF ROTIFER FEED ENRICHMENTS ON BACTERIAL COMMUNITIES OF TURBOT (*Scophthalmus maximus*) LARVAE

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Introduction

Rotifer and artemia are commonly used in larviculture as first feeds, but both are poor in essential fatty acids (EFA). To overcome this problem, a range of commercial products are currently available for live feed enrichment with EFA [1]. However, little is known about how live feed enrichment products affect the initial microbe recruitment of newly hatched larvae. In this study we attempted to determine the microbiome dynamics and their putative functioning in turbot larvae (*Scophthalmus maximus*) and rearing water in response to two distinct commercial rotifer feed enrichments.

Methods

Here, turbot larvae originated from Flatlantic (Portugal), three days after hatching (DAH), were reared in flow-through larviculture tanks during 27 days. The feeding regime was based on rotifers enriched with two different commercial rotifer enrichments (ER1 or ER2), and artemia given the same commercial enrichment (ER2; from 11 to 30 DAH). A mature culture of *Tetraselmis* sp. was added daily from 3 to 15 DAH (green water technique). Three independent larviculture tanks were used for each rotifer feed diet investigated (ER1 and ER2) and maintained under similar physicochemical conditions (temperature, salinity, pH and O_2) for 27 days. Samples for bacterial community analysis (high-throughput sequencing of the 16S rRNA gene) of the whole turbot larvae (10 DAH) and the post larval gut (30 DAH) were obtained after 7 days and 27 days of growth in the rearing tanks, respectively. The bacterial communities of rearing water and live feed [rotifer (7 days), and artemia (27 days)] were also analyzed [2]"ISSN":"0044-8486","abstract":"The stressful conditions of intensive aquaculture systems, combined with excessive antibiotic use, may have dysbiotic effects on aquaculture microbiomes and promote the spread of opportunistic pathogens. Here, we hypothesized that humic substances (HS.

Results

Our results show that rotifer enriched with different commercial enrichments (ER1 and ER2) did not significantly affect overall bacterial structure and diversity of fish larvae and water-associated bacterial communities at both sampling points (Figure 1). However, larvae fed rotifer enriched with ER1 was associated with a significantly lower relative abundance of Vibrionales at 10 and 30 DAH and an increase of Rhodobacterales members when compared with ER2 (Figure 2). Curiously, the lower *Vibrio* abundance was also followed by a strong increase in the abundance of an amplicon sequence variant (ASV) closely related to *Sulfitobacter* sp. (Roseobacter clade) [3]. Predictive metagenomic analysis also showed that KEGG orthologs related to infection disease tended to be less abundant in the treatment ER1. Interestingly, ER1 had a significant and higher survival rate (8.0 ± 0.9%) than ER2 (4.34 ± 0.89%). Overall, bacterioplankton mainly consisted of ASVs assigned to the genus *Polaribacter* and with high abundance of Roseobacter clade members.

Conclusion

Overall, this study showed that live feed enrichment formulas have the potential to affect the balance of pathogenic and antagonist microbes in larviculture. Our findings highlight the importance of analyzing the effects of rotifer enriched with different enrichment products on the dynamics of microbial communities in fish larviculture. Due to the unique structural composition of each aquaculture system, such an approach is critical for better evaluation and selection of the optimal enrichment strategy for live feed production.

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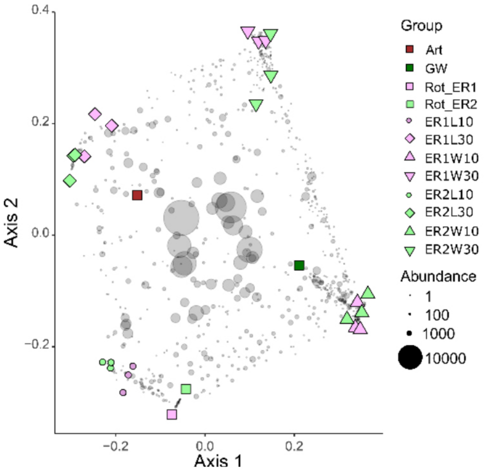

Figure 1 - First two axes of principal coordinates analysis (PCO) of ASV composition. Colored symbols are samples. Grey symbols are weighted averages scores for ASV (size is proportional to abundance). Artemia (ART), Algae (GW), Rotifer (Rot), L10/L30 (larvae 10/30 DAH), W10/W30 (water 10/30 DAH)

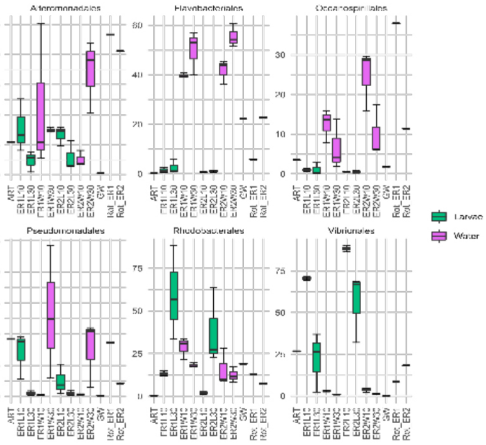

Figure 2 –Boxplot of relative abundances of most abundant orders in larvae, water and live feed (rotifer, artemia and algae) of both systems feed differently enriched rotifer (ER1 and ER2). Artemia (ART), Algae (GW), Rotifer (Rot), L10/L30 (larvae 10/30 DAH), W10/W30 (water 10/30 DAH)

Acknowledgement

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WHY IS FAO WORKING ON THE SECOND EDITION OF ITS GUIDEBOOK ON COMMERCIALLY IMPORTANT SEA CUCUMBER SPECIES OF THE WORLD FIRST PUBLISHED IN 2012?

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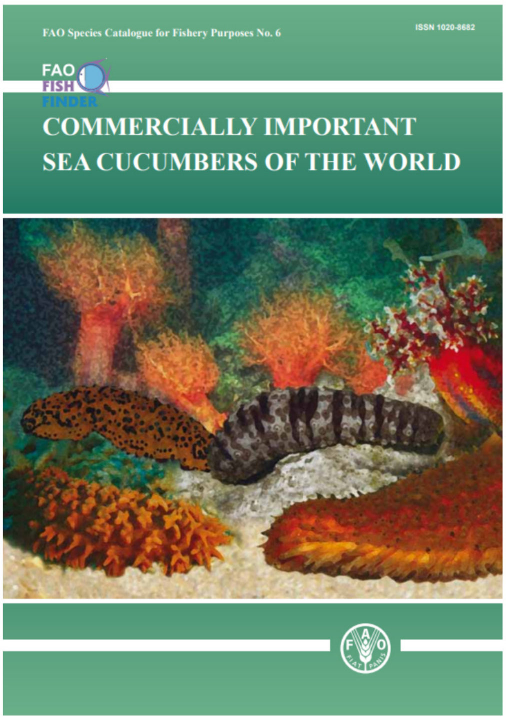
Sea cucumbers (a class of echinoderm) are a high-value traded commodity primarily consumed fresh or processed in East Asia, and now sought after by pharmaceutical and cosmetics industries. Demand for sea cucumbers has risen since the 1980s, at a rate faster than the average CPI in China. To meet the demand, extensive harvesting and trade of sea cucumbers has spread to nearly all seas and oceans, and perhaps as many as 100 species are now harvested in at least 80 countries worldwide.

Many of the species sell for over USD 100 per kg dried, and a few sell for over USD 1 000 per kg. Wild stocks in some countries have been severely overexploited, so trade expanded to new countries, and IUU fishing has risen considerably. Encouragingly, many countries have placed moratoria on their sea cucumber fisheries to allow for stock recovery or have placed stricter regulatory controls. As sea cucumbers do not form large dense aggregations, and many species coexist relatively close, the fisheries for these species are typically multispecies and scattered, with many disperse landing sites not needing specialized infrastructure, but the product usually converges on a few trade routes. This means that management of these fisheries has to rely heavily on trade regulations, including those managed through CITES. One species from the eastern Pacific is already regulated by CITES Appendix III for Ecuador. In 2019, three species from the Indo-Pacific region were placed on CITES Appendix II. More species are likely to be proposed at future CITES CoP meetings.


FAO Species Catalogue for Fishery Purposes No. 6
ISSN 1020-8682
FAO FISH FINDER
COMMERCIALLY IMPORTANT SEA CUCUMBERS OF THE WORLD

Using trade regulations as management tools requires the availability of suitable tools to identify the products of many species in trade, and these were previously lacking. In 2012, FAO published the first global identification guidebook for these resources, entitled *Commercially Important Sea Cucumbers of the World*. It illustrated 65 species, provided information to enable identification of species in live or dried forms and described the preferred habitats, fisheries, market prices and distributions. However, data were lacking at the time on numerous species from the Mediterranean Sea, West Africa, Latin America and the Caribbean. In addition, the publication did not include practical dichotomous keys for easy systematic identification of the species in fresh and/or processed product.

The 2012 guidebook has been an incredible success. More than 1 250 hard copies were distributed worldwide to target users (and a top downloaded document from the FAO Web site) and it has been cited in literature 392 times. A second edition of the guidebook is needed to provide a complete coverage of commonly exploited species worldwide, including a number of new species from the Indian and Pacific Ocean that have entered the international trade market since the publication of the first issue of the FAO guidebook. The new guidebook will contribute to the management of the fisheries for sea cucumber, particularly as it concerns knowledge generation and enforcement, contribute to reduce under-reporting of certain species in trade, and facilitate better species-specific reporting of species by exporting and importing countries. The development of this guide will thus contribute directly to the programme of work of the FAO Fisheries and Aquaculture Division by making available an essential tool for effective management of sea cucumber fisheries.

The presentation will outline the process followed in collaboration with world experts and specialist scientists to obtain new information, data and photographs of species that were not illustrated in the first edition of the guidebook. New data on retail prices from dried seafood markets will enable the guidebook to present updated market information. Recent changes to taxonomy of species will be captured in the guidebook, and novel dichotomous keys will be produced for identifying live and dried specimens.

APPARENT NUTRIENT AND ENERGY DIGESTIBILITY OF COMMERCIAL FEED INGREDIENTS WITH OR WITHOUT PROTEASE IN RAINBOW TROUT

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Introduction

One of the most important aspects in the evaluation of the effectiveness of alternative feed ingredients is the determination of its digestibility. Several alternative feed ingredients have been tested in aquaculture feeds to replace fishmeal for sustainable aquaculture. Nevertheless, imbalanced amino acid profiles, poor digestibility and palatability, and presence of anti-nutritional factors (ANFs) limit their use in aquafeeds (NRC, 2011). Therefore, one of the strategies is to supplement exogenous enzyme(s) for improving the nutrient digestibility. Among enzymes, proteases have potential use in reducing or counteracting ANFs negative impacts, such as protease inhibitors, and breaking down macromolecular proteins (Li et al., 2016). The efficacy of supplemental exogenous protease across a wide range of protein ingredients has not been previously investigated. Therefore, this study was conducted to evaluate the effects on apparent digestibility coefficients (ADCs) of dry matter, crude protein, amino acids, and gross energy of a dietary protease added to 17 different protein ingredients using rainbow trout as a model species.

Materials and Methods

In vivo digestibility was determined for 17 ingredients with and without protease supplementation (175 mg.kg^{-1}, Jefo Nutrition Inc., Quebec, Canada) fed to rainbow trout. The ingredients consisted of two feather meals, two poultry by-product meals, two meat and bone meals, sardine meal, menhaden meal, black soldier fly larvae meal, *Methanococcus maripaludis* single cell protein, soybean meal, canola meal, distiller's dried grains with solubles (DDGS), cottonseed meal, peanut meal, sunflower meal, and algae (*Spirulina* sp.) meal. A batch of test diet containing 30% test ingredient and 70% reference diet mash (combined on a dry-matter basis) was prepared and analyzed. Trout (average weight, 250 g) was used in the digestibility trial. Each of the experimental diets (reference and 34 test diets) was fed to two replicate tanks of fish in a completely randomized design to apparent satiation. Feces were expelled from each fish using gentle pressure on the lower abdomen of fish. ADC of diets and ingredients, for dry matter, protein, amino acids and energy were calculated using the following formula described by Bureau et al. (1999). Apparent digestibility was calculated using fecal material pooled from 30 fish/tank, and all data are expressed as the mean ± standard error of the mean (SE). Data were subjected to a Student's t-test to test for protease effect using SPSS Version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

ADC of dry matter for rainbow trout ranged 51.0–86.6% for animal products and single cell protein and 33.1–70.1% for plant products without protease supplementation. ADC (without protease supplementation) of protein and energy ranged from 55.4–84.5% and 58.1–90.2%, respectively, for animal products, and 70.0–83.8% and 32.9–76.0%, respectively, for plant products. Supplementation with the commercial protease (175 mg protease/kg of diet) resulted in ingredient-specific ADC increases for dry matter, energy, cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, alanine, aspartic acid and glutamic acid, with most ingredients having improved digestibility of at least one amino acid. Protease supplementation had the most profound improvement on ADCs for soybean meal, including dry matter and the majority of individual amino acids.

Conclusion

Supplementation with the exogenous protease resulted in ingredient-specific ADC increases for dry matter, energy, cysteine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, alanine, aspartic acid and glutamic acid, with most ingredients having improved digestibility of at least one amino acid. Protease supplementation had the most profound improvement on ADCs for soybean meal, including dry matter and the majority of individual amino acids. Overall, although the degree of improvement in digestibility varied among ingredients, this research demonstrates the benefit of the evaluated protease supplementation on the digestibility of feed ingredients commonly used in rainbow trout and other commercially cultured fish feeds.

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IMPACT OF OMEGA-3 DIETARY SUPPLEMENTATION ON LARVAL SKELETAL DEVELOPMENT, BONE MINERALISATION, AND OXIDATIVE STRESS IN A FAST-GROWTH SPECIES: *Argyrosomus regius*

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Introduction

Meagre is one of the most promising species for the diversification of European aquaculture. However, nutritional requirements for early larval stages are not sufficiently known. Due to their fast growth rate, high demand for essential fatty acids (EFA) is expected 1there is a lack of information on nutrition along larval development. Importance of highly unsaturated fatty acids (HUFA. Among them, Docosahexaenoic acid (DHA) is crucial for marine fish larval growth and development. Dietary deficiencies have been associated with increasing skeletal malformations, whereas high DHA levels have produced controversial results. The oxidative potential of elevated DHA levels has been related to deleterious results when inadequate amounts of antioxidants are included. 2but high DHA levels have produced controversial results that could relate to the oxidative status of fish tissues in the different reports. In the present study, gilthead seabream (Sparus aurata. To study optimal levels of DHA supplementation and the most optimal level of antioxidant, meagre larvae were fed diets presenting increasing levels of DHA. In addition, the impact of the antioxidant level was examined by employing a diet with a deficient level of vitamin E. Furthermore, a commercial diet, presenting the lowest level of DHA was used as Control to compare larval culture success. The results of this comparative study including assessment of growth, skeletal development, and presence of skeletal deformities will be discussed together with the analysis of antioxidants enzymes activity. These insights will contribute to our understanding of the biochemical mechanisms employed by DHA in controlling skeletal metabolism, offering important knowledge to improve dietary formulations.

Material and methods

Fish larvae of A. regius from the same spawn were distributed by 9 rearing tanks of 300 L from 0 DAH to 42 DAH covering the period from the beginning of skeletogenesis, until the end of skeletal ossification. The larvae were fed with three different experimental diets (live preys and inert micro-diets) which presented increasing levels of DHA: CONTROL, MD with 2.6, and HD with 3.6 (% DW) with the adequate level of antioxidant for each level of DHA respectively. Furthermore, a fourth diet (MD-VE) with 2.6 of DHA (% DW) but a deficient level of antioxidant Vitamin E. All the emulsions and diets were produced by SPAROS, including the diet employed as a control for culture performance named WINFAST. Fish larvae were periodically sampled (at 12, 22, 32, and 42 DAH), photographed to assess larval growth, and stained with Alcian Blue and Alizarin S to assess the coefficient of mineralization and frequency of anomalies. Culture performance was further characterized by calculating daily growth (DGI, %), and specific growth rate (SGR, %). Besides, at 14, 28, and 42 DAH larvae were sampled for biochemical analysis including antioxidants enzymes activity (CAT, SOD, and GPX) and lipid composition of larval tissues. All the remaining larvae at the end of the trial were counted to determine the survival rate.

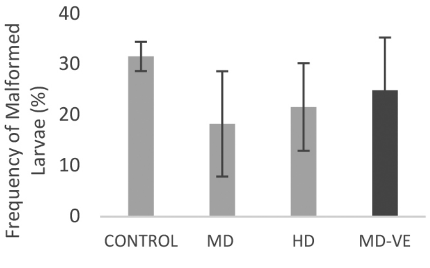

Figure 2. Frequency of malformed larvae per treatment at 42 Days After Hatching (p<0.05).

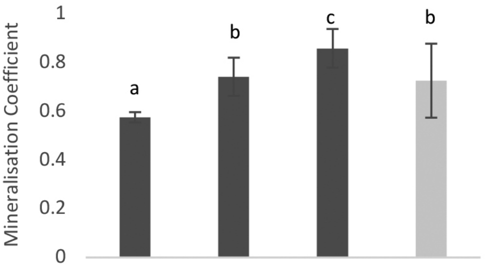

Figure 1. Coefficient of mineralization per treatment at 42 Days After Hatching (p<0.05).

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Results and Discussion

Fish larvae promptly accepted the experimental diets supplemented with ω3 fatty acids and survival was not affected by dietary treatment. However, growth performance in larval cultures seemed to improve by the supplementation with n-3 PUFAs, being the larvae fed with the HD diet exhibited the highest growth at the end of the trial. However, no linearity was observed between growth and DHA dietary level since meagre larvae fed MD diet with the intermedium level of n-3 supplementation exhibited lower growth at 42 DAH than the CONTROL. Similar results were also observed regarding DW. However, no significant differences were observed among treatments regarding SGR, and only HD presented a significantly higher DGI than the rest of the treatments. When growth performance was compared among the two intermedium diets with different content of Vitamin E, no differences were observed in TL whereas the lower level of Vitamin E presented in diet MD-VE improved DW significantly at the end of the trial.

Regarding the progress of ossification, the results reflected a similar tendency to growth, where the diet with the highest content of ω3 fatty acids presented the fastest results. However, contrary to growth the MD presented a faster rate of mineralization than the CONTROL diet, denoting that the improvement in the ossification was linearly correlated to the n-3 DHA dietary content. Moreover, no differences were observed between the diet MD and MD-VE denoting that Vitamin E levels didn't affect mineralization progress. However, regarding the activity of the antioxidant enzymes in larval tissues, some differences in GPX total and GPX Se-Dependent were observed when treatments were compared regarding both conditions, the level of DHA in diet and the level of Vitamin E.

Despite no differences were observed a 42 DAH regarding the frequency of malformed larvae, some differences were observed during the trial regarding the type of malformities and the frequency of anomalies by body region. Therefore, we can conclude that the supplementation of high levels of DHA n-3 fatty acids improved growth and accelerated larval skeletal development in this specie without increase significantly the percentage of malformed individuals. However, the different dietary levels of DHA and the level of Vitamin E in the diet affected differently the type, the region, or the severity of malformations during the trial.

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Acknowlegments

This work is part of a project that has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 766347.

INTERACTION BETWEEN DIETARY OMEGA-3 FATTY ACIDS AND FILLET COLOR IN ATLANTIC SALMON (*Salmo salar*): A TRANSCRIPTOME STUDY

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Introduction

Fillet color is one important trait defining flesh quality in Atlantic salmon. Dietary supplementation of carotenoids, mainly astaxanthin, is a common strategy to increase fillet pigmentation and its retention in tissues appear to be influenced by nutritional factors. Recent studies have shown a link between omega-3 levels in the diet, particularly EPA and DHA, and fillet color in Atlantic salmon (Bou et al., 2017, Lutfi et al., 2022). Nevertheless, the specific regulation of factors determining the deposition of astaxanthin in different tissues and the fillet color is still not clearly elucidated. The present study aims to evaluate the potential relationship between omega-3 fatty acids and fillet color by looking into transcriptional and metabolic regulations in the three main tissues involved in astaxanthin metabolism (muscle, liver, and intestine).

Materials and methods

Atlantic salmon from Mowi Genetics were distributed across six sea cages (5x5m^2) located at the Mowi's research station in Averøy (Norway) and fed 2 different diets based on a common low marine base formulation (Skretting ARC) coated with either a high vegetable oil/low fish oil mix (Control diet, **CT**) or an omega-3 LC-PUFA-rich microalgal oil from Veramaris® (Omega3, **Ω3**). At the final sampling, all fish from each sea cage were randomly taken and 10 fish from each dietary group were selected for **High** (5 fish) and **Low** (5 fish) fillet color based on VIS/NIR spectroscopy. The main purpose of this selection strategy was to create high contrast among the different conditions (**CT-High, CT-Low, Ω3-High and Ω3-Low)** in order to establish potential correlations between results obtained by transcriptomics, metabolomics and chemical analyses. Samples of muscle, liver, and intestine were taken for different analyses. The present study was conducted within the AquaIMPACT project (H2020 BG2018-818367) and analyses were co-financed by Nofima's internal projects.

Results

The microarray results showed that the "pigment selection" had the most significant impact, particularly in the liver (Fig.1A). The PCA plots display a clustering of samples with similar gene expression profiles in the same area. Scores of samples belonging to either high pigment or low pigment, were located together respectively and slightly influenced by the dietary treatments, particularly within the low pigment group (in green). In this regard, 23.94% (muscle), 28.17% (liver) and 24.94% (intestine) of the variation was explained by the first principal component (x-axis), which distributed the samples along the axis from high pigment, in the left quadrants, to low pigment, in the right quadrants (Fig. 1B-D). This distribution is again particularly evident in the liver. Moreover, the liver showed the largest number of unique differentially expressed genes (315) compared to intestine (139) and muscle (144) (Fig. 1E). However, no differential regulation of the common genes (9) was observed in the different tissues. Further analyses comparing common genes in one-by-one tissues (i.e. intestine vs. muscle) will be conducted.

Discussion and conclusions

Overall, our results showed a clear effect of the fillet pigment selection on the transcriptional regulation of all tissues and particularly in the liver (also with the highest number of differentially expressed genes) highlighting the potential involvement of this tissue in regulating astaxanthin deposition and fillet color. Moreover, diets also had a significant differential effect within the high and specially the low pigment group supporting the idea that dietary omega-3 fatty acids also influence fillet color. Additional integrative analyses including transcriptomics, metabolomics and fatty acid composition analyses are currently being conducted.

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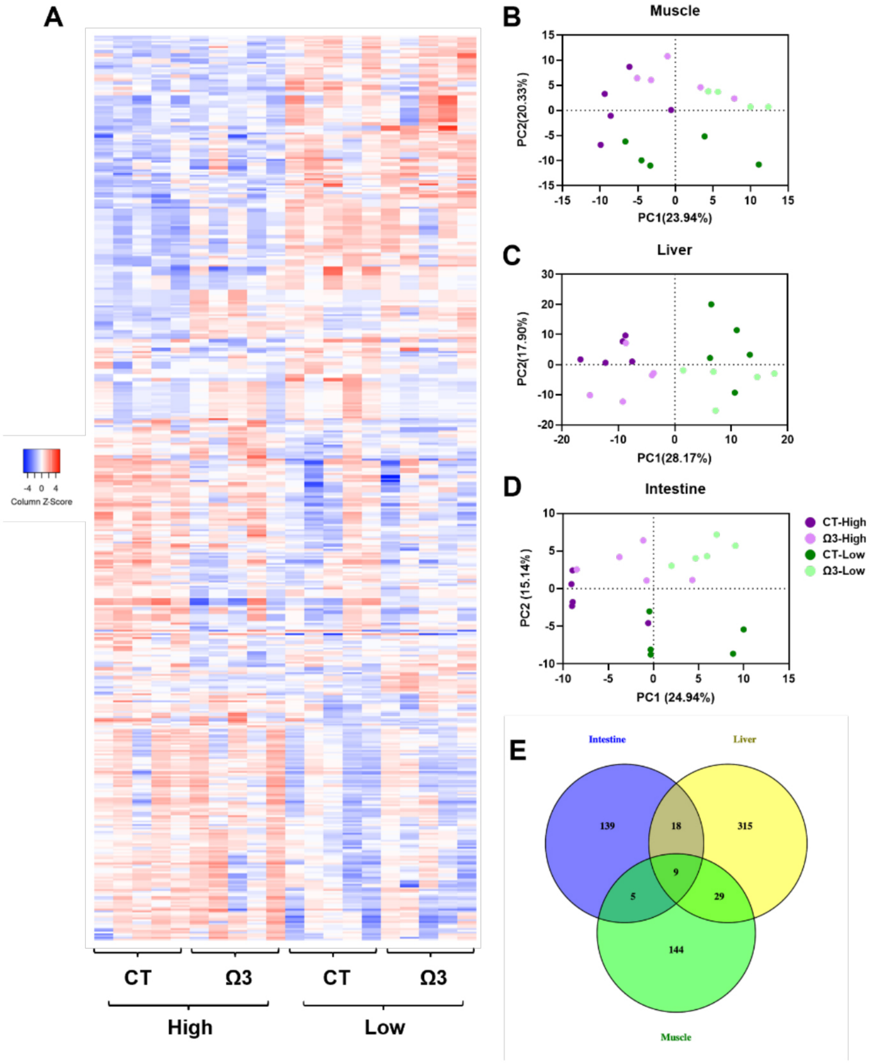

Figure 1. Microarray-based gene expression analysis. **A**, Heatmap plot of microarray data from liver. Blue indicates decreased expression and red indicates increased expression. **B**, **C**, and **D**, Score plots from PCA models calculated on the relative gene expression values of muscle, liver, and intestine respectively. **E**, Venn diagram of the microarray results in muscle, liver, and intestine.

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THE EFFICACY OF USING PINE (*Pinus massoniana*) POLLEN AS AN ALTERNATIVE TO SYNTHETIC STEROIDS IN PRODUCING MONOSEX MALE NILE TILAPIA (*Oreochromis niloticus*, L.)

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Synthetic sex steroids are widely used to produce all-male stocks in Nile tilapia (*Oreochromis niloticus*). However, there is a growing concern of the use of synthetic steroid hormone in fish as food. We investigated the efficacy of a natural sex hormone as an alternative to synthetic steroids in producing monosex male tilapia. Two-day old fry was fed with pine pollen powder at three different concentrations (0.5, 1.0, 1.5%) for 28 days and subsequently reared for three months to observe the efficacy of pine pollen as well as to optimize the concentration for sex reversal. As positive control, same-aged fry of three different commercial tilapia hatcheries was fed with synthetic steroid, 17-α-methyl testosterone treated feed (60 mg/kg) for the same duration. Before analyzing the phenotypic sex, the fish were reared on untreated commercial feed for 90 days in a separate tank. The 1.0% pine pollen treatment yielded a mean of 92.50% males among the progeny, higher than the mean (89.94%) obtained from synthetic steroid treatments. This result demonstrates that pollen of pine could be an effective compound for the direct masculinization of Nile tilapia. We also obtained a significant difference ($p<0.05$) in the growth performance and survival rate of tilapia using dietary allotment of pine pollen. Our work suggests the feasibility of this compound as an eco-friendly method of masculinization of tilapia and can be tried in commercial aquaculture practice.

EFFECT OF DIETARY PHOSPHORUS LEVELS, WATER PHOSPHORUS LEVELS AND THEIR COMBINATION ON GROWTH PERFORMANCE AND PHOSPHORUS UTILISATION IN NILE TILAPIA

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Introduction

Replacing fishmeal with plant ingredients coincides with decreased bioavailability of dietary phosphorus (P) in fish feeds, since phytate bound P is poorly absorbed by fish. In aquafeeds that contain more plant-based ingredients, inorganic P needs to be supplemented in order to fulfil the P requirements for growth. However, using P from (mined) phosphate rock deposits is unsustainable, given that natural reserves of phosphate are limited (Prabhu et al., 2013; Obersteiner et al., 2013). It is essential that sufficient levels of bioavailable P are present in aquafeeds, as P plays a fundamental role in the synthesis of ATP and nucleic acid, and the formation of bones and scales (Fontagne et al., 2009). By improving the bioavailability of phytate bound P from plant ingredients, the dependency of aquaculture on inorganic mined P sources and the level of dietary P in aquaculture effluent can be reduced. In recirculating aquaculture systems (RAS), minerals can accumulate in the water (including P), which can lead to waterborne mineral uptake by fish. Whether or not fish take up P (and other minerals) from the water is most likely related to the amount of available minerals in the feed, as well as the concentration of available minerals in the water (Rodehutscord et al., 2000; Prabhu et al., 2017). When denitrification occurs, phytate can be partly broken down (when faecal waste is used as carbon source) and P becomes soluble in water (Goddek et al., 2016; Goddek et al., 2018); this, in combination with low water exchange, can lead to accumulation of P in the water. The use of phytase in aquafeeds is getting more attention as a way to break down phytate and improve the dietary P availability (Kumar et al. 2012). The presented experiment evaluated two potential ways to increase the P availability in aquafeeds (high in phytate): 1) increasing the dietary availability of P through the diet with the use of phytase, and 2) increasing the P availability in the water using a system low in water refreshment with the use of a sludge denitrification reactor. With the experimental setup, the contribution of water born P uptake to growth was determined, and whether the effect of water born P uptake is dependent on the availability of dietary P.

Materials and Methods

A plant-based diet high in phytate and non-starch polysaccharides (carbon source denitrification) was used. The effect of available dietary P level was tested by supplementing phytase (PHY) versus a control without phytase (CON). The contrast in water available P was achieved by using a system with a high water refreshment (HR, low in water P) versus a system low in water refreshment (LR, high water P), using RAS with a denitrification reactor. The four treatments were studied over a period of 56 days according to a 2 x 2 factorial design. Tanks in the HR system were stocked with 30 and the LR with 40 Tilapia (mean initial weight 30 g). Fish were restrictively fed twice a day (aimed feeding level: 17 g/kg$^{0.8}$). For each tank growth performance was measured and the P utilisation calculated/estimated. The digestibility and the effect of phytase on the digestibility were only tested in the LR system (the faecal waste in the HR system was used as carbon source for the denitrification reactor).

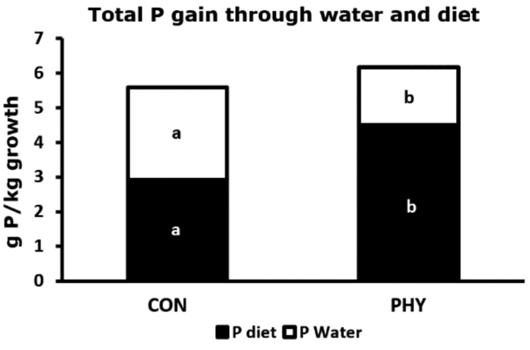

Figure 1. Mean minimum phosphorus (P) gain from the water and maximum P gain through the diet (g/kg growth) in the low water refreshment treatments (CON, control diet; PHY, diet supplemented with phytase). Bars with different lower-case letters are significantly different (p<0.01).

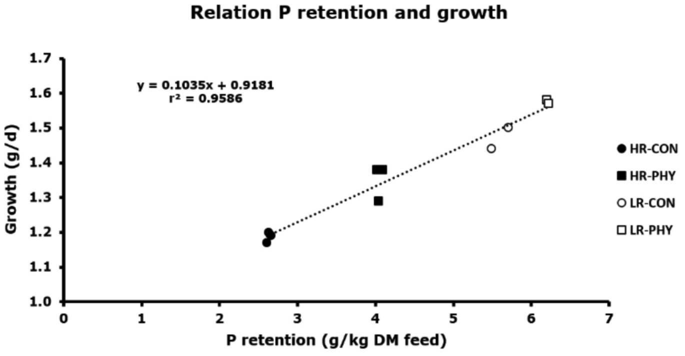

Figure 2. Correlation between P retention (g/kg DM feed) and growth (g/d). P retention in HR-CON and LR-PHY were estimated as 90% of the available P. HR-CON, high-water refreshment + control diet; HR-PHY, high-water refreshment + phytase diet; LR-CON, low-water refreshment + control diet; LR-PH, Low-water refreshment + phytase diet

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Results

Phytase improved the dietary P availability with 56% (to 4.5 g P/kg DM), in both water P levels, resulting in higher growth (g/d). Increasing the water P levels using a denitrification reactor, resulted in higher growth (g/d). The effect of phytase on growth (SGR, %/d), was larger in the low water P treatment (interaction effect, $P<0.05$). For both diets, water P uptake occurred. However, the water P uptake was larger for the control diet, low in available P (figure 1; $P<0.05$). For the control treatment in the high water P treatment (HR-CON), at least 47% of the P retained was taken up from the water. Improving the P retention by increasing the P availability in the diet and/or increasing the water P levels, correlated with improved growth (figure 2).

Conclusion

Water P utpake under high water P conditions can partly compensate a P deficiency for P retention. The effect of increasing P retention on growth suggest that the P availability was limiting growth (figure 2) for the low water P and low dietary P levels. This study shows that at least 6.5 g P retention is needed per kg weight gain to sustain maximal growth. Meaning the P availability in the diet should be above 6.5 g P/kg DM when the diet is the only P source (and has a FCR of 1).

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IMPACT OF *Ulva lacinulata* ON THE MICROBIOME OF ABALONE-ULVA IMTA SYSTEMS

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Introduction

Several land-based abalone, *Haliotis midae*, farms in South Africa practice integrated multi-tropic aquaculture (IMTA) by growing *Ulva lacinulata* in abalone effluent. The *Ulva* serves as a biofilter, allows partial recirculation (saving on pumping costs), and the co-produced *Ulva* is often used as supplementary feed. Dietary seaweed supplementation has also previously been shown to improve feed consumption, growth and health of cultured abalone (Naidoo et al., 2006; Mulvaney et al., 2013; Kemp et al. 2015; Bansemer *et al*., 2016). Despite these benefits, biosecurity concerns associated with water recirculation and use of effluent-grown *Ulva* as feed are preventing wider adoption of this technology on abalone farms in South Africa. To better understand potential benefits and risks associated with IMTA, this study characterized the microbiome associated with seawater, *Ulva* and abalone from integrated abalone-*Ulva* systems.

Materials and Methods

Ulva and seawater samples were collected across seasons from four *Ulva* paddle-raceway systems receiving abalone effluent water (AEW), and the microbiome was characterized using culture-dependent and -independent techniques. *Ulva* grown in a raceway receiving seawater (SW) directly from the adjacent coastline was included as a control. Culture-dependent approach used three selective media to assess changes in the abundance of specific bacteria, whereas the non-culture-based approach used next generation sequencing (NGS) and downstream bioinformatics analysis to describe the microbiome. A separate controlled laboratory trial was conducted to assess the effects of *Ulva* and specific components of *Ulva* on abalone gut microbiome. Abalone were fed four isonitrogenous diets consisting of formulated feed (AB), and AB supplemented with dried *Ulva* (10% w/w; AB10U), Ulvan (1% w/w; AB1U), and glucuronic acid (0.1% w/w; AB0.1U). Abalone fed fresh IMTA-grown *Ulva* (FU) and a combination of FU and AB served as controls. The bacterial microbiome was characterised by sequencing the V3-4 hyper-variable region of the 16S rRNA gene. NGS was performed on an Illumina MiSeq sequencing platform, sequence data assessed using QIIME2 (Boylen et al., 2019), reads mapped against the SILVA 16S rRNA database (Quast et al., 2013) and summarized taxonomic abundance at different hierarchical levels were assessed using MicrobiomeAnalyst (Dhariwal et al., 2017).

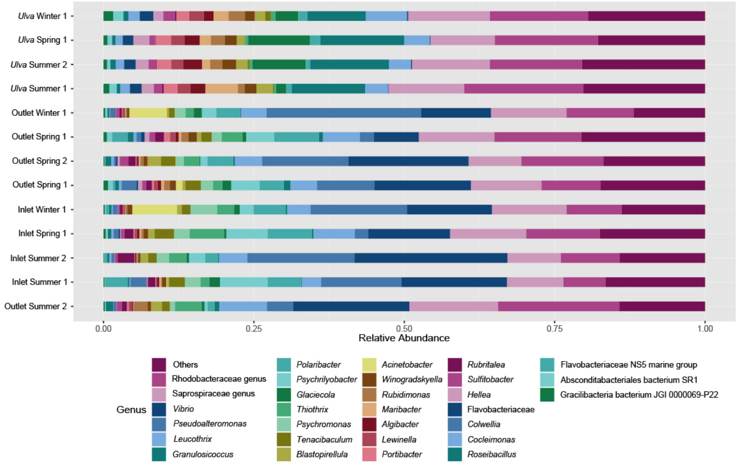

Fig.1. Relative ASV abundance (%) of inlets and outlets of *Ulva* raceways and *Ulva* itself (n = 60) at genus level, across seasons where ASV abundance is indicated for the 30 most abundant ASVs.

(Continued on next page)

Results and Discussion

Culturable bacterial numbers were significantly higher ($p<0.05$) in water than on the *Ulva* collected from each *Ulva* raceway system, with a reduction in bacterial abundance recorded from the inlet to outlet of each raceway. In the culture-independent approach, the genera *Vibrio* and *Psuedoalteromonas* were shown to be more prevalent in water samples than on *Ulva*, decreasing in abundance from the inlet to outlet of each *Ulva* raceway (Fig.1). Conversely, genera such as the *Granulosicoccus* and *Glaciecola* were more prevalent on *Ulva.* Across all seasons, *Ulva lacinulata* or its associated microbiome had a modulatory effect on the microbiome of the *Ulva* paddle-raceway systems.

Non-metric multidimensional scaling (NMDS) analysis of microbiome data revealed that abalone fed FU diets, and its components, produced significant associations in their intestinal bacterial communities, suggesting specific bacterial species are selected for and are associated with the digestive tract of abalone fed components of *Ulva*. Using DESeq2, several differentially abundant amplicon sequence variants (ASVs) were identified across dietary treatments, with various bacterial genera, including members of the genus *Vibrio* found to be less abundant in the gut of abalone fed FU supplemented diets compared to AB alone.

Conclusion

We demonstrated that inclusion of *Ulva lacinulata* in IMTA systems can beneficially alter the microbiome of the water and aquacultured products grown within the system. The findings from this study are providing critical information on biosecurity of IMTA systems, species health and system health that may promote broader uptake of these more sustainable aquaculture production technologies.

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MODELING AQUACULTURE SUITABILITY IN A CHANGING CLIMATE

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Introduction

Climate change-induced ocean warming is a major driver of distributional changes in global marine biodiversity (Cheung et al., 2009; Poloczanska et al., 2013; Chaundhary et al., 2021). The cascading effects brought about by anthropogenic climate change will impact the way in which humans utilize marine resources in the near future. Already the annual global demand for seafood has risen steadily since 1960 and this need is only projected to grow further, driven by increasing global populations and shifting economies (FAO, 2016, Naylor 2021). World aquaculture production has increased every year since 1990 (FAO 2020), while at the same time, up to 89% of the world's capture fisheries are fully exploited, overexploited, or have collapsed (Froese et al. 2012). Continued expansion of aquaculture to meet global demand is contingent upon climate adaptation and forward-thinking development. We show that models have an important role to play in guiding and predicting future highly productive aquaculture regions. This study seeks to create a multi-dimensional metric incorporating environmental, economic, and demographic shifts to identify regions of rapidly changing aquaculture suitability under different climate change scenarios.

Modeling approach

While species distribution models (SDMs) have long been used to assess the impact of climate change on populations, climate analogue modeling provides a systems-level approach to identifying likely disruption due to climate change. Climate analogues, areas that provide in future climatic conditions similar to those in a given location in the present, are already being used to determine necessary shifts in agriculture to track productive climates (Ramírez Villegas et al. 2011). Climate analogue development uses environmental parameters (such as temperature, salinity, current velocity, and dissolved oxygen) relevant to species identified in our aquaculture inventory. By carrying out this analysis on a global scale for aquaculture species and future climate change scenarios, such as RCP 2.6, 6.0, and 8.5 as described by the IPCC (IPCC 2014), we can identify areas likely to lose or inherit climates traditionally associated with aquaculture production of a wide variety of species. Previous such aquaculture studies have ignored certain groups, such as seaweeds, or extreme climate change scenarios, such as RCP 8.5, the high emissions scenario. Particular emphasis is placed on previously unidentified "bright spots" (Bennett et al. 2016, Queirós et al. 2021) where, based on models, aquaculture is projected to be more viable in the future, even under intense climate change scenarios.

Research outcomes

Current areas of aquaculture suitability, where optimal environmental conditions and species' physiological tolerances intersect, will shift to varying degrees within the different climate change scenarios (Vaz et al., 2021). Determining areas of future aquaculture suitability not only depends on the environmental variables of available areas and the species that will be cultivated but also on socioeconomic factors. Infrastructure, zoning legislation, seafood demand, farmed seafood price, and fish meal and fish oil supply are among the parameters which will further limit the conditions for aquaculture suitability (Cisneros-Montemayor et al., 2021; Oyinlola et al., 2021). This research will present alternative and under-utilised modeling tools to build on previous studies (Froelich et al., 2018; Oyinlola et al. 2018), identifying future locations ideal for aquaculture development. Additionally, this research will explore a broader definition of aquaculture suitability to include socioeconomic factors. The described modeling framework will produce estimates of future aquaculture suitability relative to the present, with an emphasis on areas experiencing exceptionally high or low climate displacement, as well as on those predicted to experience novel climate regimes. The results of this modeling will be a projection of the geographic distribution of suitable environments for each species, which can be used to identify current aquaculture areas at risk to become non-viable, as well as prospective new areas for at-sea culture.

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OPTIMISING SEEDING AND PRODUCTION METHODS FOR COMMERCIAL KELP CULTIVATION

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The cost of seeded material, either through on-site or outsourced production, is often seen as a constraint to the development of seaweed aquaculture in Europe. The mechanical application of fertilised gametophyte fragments to cultivation materials with the use of a hydrocolloid binder provides a low-cost seeding solution that is rapidly scalable. However, "direct-seeding" is not widely regarded as a reliable method to seed cultivation materials and guarantee adequate yield performance. High operational costs, combined with inefficient farm designs, generate further economic challenges for cultivators, narrowing profit margins and intensifying current operational and market uncertainties.

To improve the reliability of direct-seeding protocols, several controlled mesocosm experiments were combined with flume studies to assess binder performance under controlled flow conditions. New binder chemistries, seeding protocols and cultivation materials were tested to optimise conditions which promote successful attachment of juvenile kelp. High performing treatments were validated in the field following deployments at two cultivation sites and at two time points allowing for a comparison of yield and seeding density to be made against existing direct seeding protocols.

Deployment strategies that improve crop performance and farm efficiency were tested at an experimental seaweed farm located on the west coast of Scotland. Optimum stocking densities (2 - 0.5 $m^{-1}m^{2}$) were assessed for two commercially important species of kelp (*Saccharina latissima* and *Alaria esculenta*). Similarly, the optimum cultivation depth following deployment was also established and yield and morphology data linked to Photosynthetically Active Radiation (PAR) across 9 cultivation depths ranging from 0.1-4m water depth.

In this presentation I will compare results from single-step and two-step seeding protocols which demonstrate the vulnerability inherent with viscosity-based binder solutions used to date and discuss improvements we have made to alter binder chemistry to protect sporophytes throughout the attachment phase (ca. 14 days). I will provide an overview of our approach to the production and delivery of direct seeded materials which is tailored to customer requirements and improved circularity of cultivation materials. Finally, I will share results from trials that highlight deployment strategies (optimal stocking density and variable depth systems) that improve crop performance and farm efficiency.

ASSESSING THE BEST FEEDING STRATEGY IN GSB AND ESB TO MODULATE FILLETS' N3/N6 FATTY ACIDS RATIO

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Introduction

Fish consumption is widely recognized as the healthiest way to ensure a proper intake of n3 long chain highly unsaturated fatty acids (LC-HUFA) such as EPA (C20:5n3) and DHA (C22:6n3). The benefits of these FA for human health are well known and relate with fetal, brain and vision development, cardiovascular function, cognition, or depression disorders. The European Food Safety Agency (EFSA) even recommends for European adults a daily intake of 250-500mg of EPA and DHA based on cardiovascular risk consideration. Aquaculture fish, whose global consumption has been rising every year, will increasingly play a crucial role in delivering healthy n3 LC-HUFA to the population.

In the last decades there was an important shift in aquafeed recipes towards higher inclusion levels of vegetables, with an impact on fillets n3 and n6 FA pattern and, consequently, on their nutritional value for human consumption. It is known that n3 and n6 FA are precursors of signaling molecules with opposite effects, namely anti-inflammatory and pro-inflammatory, respectively. Gilthead seabream (GSB) and European seabass (ESB) have limited or no ability to synthesize n3 HUFAs from C18 FA [such as LA (C18:2n6) or ALA (C18:3n3)] present in vegetable ingredients. Therefore, the feed must be a direct source of EPA and DHA. The strategy to enrich fish fillets in n3 FA has been the adoption of finishing diets, for 120-150 days pre-harvesting, with greater inclusion levels of fish oil (FO), to compensate for the low intake of EPA and DHA throughout the cycle and to allow for their restoration in the fillet. Switching to a diet rich in fish oil during the late growout is likely to adjust fish FA profile for better n3 FA content. However, all lipid sources consumed during the growout period will influence the final lipid composition and fat distribution in the tissues of farmed fish.

Going further on this topic, i.e., considering not only the amount of EPA and DHA in the fillet but also the ratio n3/n6, the goal of this work was to assess the best nutritional strategy for GSB and ESB, for both species to deliver more interesting and healthy fillets for the final consumer.

Material and methods

GSB and ESB batches from 2018, 2019 and 2020, raised in a Spanish industrial land production facility, were divided in two groups considering the nutritional strategy adopted in terms of lipid sources in the feed: FORO) a mixture of fish oil and rapeseed oil or FO) 100% FO (high in n3-LC HUFA). FORO diet presented 6g EPA+DHA/100g fat and a n3/n6 ratio of 0,9, whereas FO diet presented 26g EPA+DHA/100g fat and a n3/n6 ratio of 3,5. The experiment started in September 2020 and lasted for 12 months. Initial, intermediate, and final samplings were performed, each consisting of 5 individuals per group; all fish were weighted and filleted; a composite muscle sample was therefore analyzed for the fat content and for the FA profile by Mérieux NutriSciences (Portugal).

Results

The fish sampled varied in size from 94 to 771g in GSB and from 299 to 1372g in ESB. As expected, fillet fat content positively correlated with fish body weight in both species ($R^2>0,5$). The season effect in GSB in terms of fatty acid deposition in the fillet was very clear, with the increase in fat during the summer months and its loss during the winter season. During fat loss process, EPA and DHA are preferentially oxidized comparing to n6 FA as their proportion in fat decrease. Nonetheless, EPA and DHA in total fat amount tends to be above total n6 FA. Feeding with 100% FO diets allowed GSB to go from a n3/n6 ratio of 0,9 in April to 1,4 in September, whereas the usage of FORO diet allowed to go from 0,9 to 1,2. In seabass there was a clear response of fillet lipid profile to the diet throughout the cycle, without a season effect. The intake of 100% FO diet allowed for seabass to shift to a higher proportion of EPA and DHA in the fat over n6 FA, increasing therefore the ratio n3/n6. In fact, with a FO-based diet, seabass was able to attain 1,1 of ratio n3/n6 whereas with the FORO-based diet seabass was in average around 0,9. Both GSB and ESB showed EPA and DHA content between 500 and 1500mg/100g fillet, meaning that 100g of all sampled fish were enough to meet the recommended daily intake by EFSA of these FA.

Conclusions

This experiment conducted in industrial conditions, aimed at understanding the modulation of FA in GSB and ESB muscle depending on feed lipid sources, showed that GSB is likely to improve the characteristics of its fillets towards higher EPA and DHA levels and greater n3/n6 ratio during the summer period, whereas ESB may need a continuous feeding on selected high in n3-LC HUFA FO diets to attain this goal.

ARE ARA, DHA, AND CARBOHYDRATES KEY PLAYERS TO MODULATE THE IMMUNE STATUS OF GILTHEAD SEA BREAM JUVENILES?

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Introduction

The aquafeeds industry for marine carnivorous fish such as gilthead sea bream (*Sparus aurata*) is increasing the dietary incorporation of plant feedstuffs and vegetable oil (VO), leading to an increase in the dietary content of carbohydrates and a reduction of long-chain polyunsaturated fatty acids (LC-PUFA). However, VOs are devoid of LC-PUFA such as docosahexaenoic (DHA) and arachidonic (ARA) acids, which are key protagonists of the immune response as precursors of eicosanoids and in the induction of immune-related transcription factors. Marine fish are not able to meet their ARA and DHA requirements by endogenous synthesis from their C-18 precursors, thus it is important to understand how novel diets rich in plant feedstuffs and VO may affect gilthead sea bream immunological status, particularly when challenged by a pathological agent.

Material and methods

Groups of 20 gilthead sea bream juveniles (initial body weight = 47.5 g) were distributed in 12 tanks and fed four isoproteic (47%) and isolipidic (18%) diets with high or low gelatinized starch (HS or LS diets) content and dietary ARA/DHA ratios of 2.3/03 (ARA diets) or 0/2.6 (DHA diets). The growth trial lasted for 84 days and then the blood and plasma of 9 fish per diet were sampled. Thereafter, 24 fish from each dietary treatment were randomly selected and intraperitoneally injected (i.p.) with 0.6 mL of killed *P. damselae* subsp. *piscicida* (*Phdp*) ($1x10^8$ CFU mL^{-1}) or PBS (control group). Blood and plasma of these fish were sampled 4 and 24 hours after i.p..

Results

At the end of the growth trial, it was observed that DHA-rich diets increased red blood cell (RBC) numbers, hemoglobin, and plasmatic nitric oxide (NO) levels, antiproteases, and alternative complement activities. Fish fed the ARA/HS diet had increased thrombocytes and plasmatic bactericidal activity against *Vibrio anguillarum* compared with fish fed ARA/LS diet.

At 4 hours after i.p., fish fed with the DHA diet presented increased RBC numbers within HS groups as well as hematocrit, and thrombocytes numbers. Fish fed the ARA diets had increased total immunoglobulins and plasmatic bactericidal activity against *Vibrio anguillarum*. Within the ARA groups, fish fed the HS diet had increased white blood cell (WBC) and lymphocytes numbers, and alternative complement activity.
At 24 hours after i.p., fish fed the ARA diets had higher RBC, WBC, and thrombocytes numbers and increased plasma IG concentration and peroxidase activity. Compared with the LS groups, fish fed with the HS diets had higher plasmatic NO content and bactericidal activity against *Phdp* and lower proteases, antiproteases, and bactericidal activities against *Vibrio anguillarum*.

Conclusions

DHA-rich diets seemed to enhance the immune status of gilthead sea bream juveniles after the growth trial while ARA-rich diets seemed to improve the immune response after a *Phdp* challenge. The effect on the immune response of dietary carbohydrates and its interaction with dietary DHA and ARA were inconclusive. Further research is required to establish the optimum dietary ARA/DHA ratio for optimization of the immune response of gilthead sea bream juveniles.

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NUTRITIONAL EVALUATION OF SOME ECONOMICALLY IMPORTANT MARINE AND FRESHWATER SHELLFISH SPECIES OF BANGLADESH

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Molluscs are the most important resources among all the seafood items in South-East Asian countries. However, very little information available on nutritional value of molluscs in these regions. In this study, we evaluated the 7 economically important species of molluscs in terms of proximate composition, amino acids profile, fatty acids profile, cholesterol and heavy metal contents in the bivalves (mussels, oysters, clams and cockles) and univalve (snail) collected from freshwater and marine environments of Bangladesh. The results of the proximate analyses revealed that significantly higher amount of crude protein contents were present in marine water oysters, clams and cockles (59.3 ± 0.3 to 75.4 ± 0.2%) than the freshwater mussels and snail (36.9 ± 0.4 to 49.6 ± 0.6%) on dry matter basis. However, carbohydrate contents were significantly higher in freshwater mussels and snail (30.2±± 0.9 to 57.3±0.2%) compared to the marine water bivalves (8.1 ± 0.4 to 20.2 ± 0.6%). Crude lipid contents were ranged from 2.5 ± 0.2 to 11.2 ± 0.1% and ash from 11.4 ± 0.1 to 16.8 ± 0.6% among the bivalves and snail species. The amino acid contents were comparatively higher in marine water bivalves than their freshwater counterparts. Saturated fatty acid contents were found to be higher in marine water bivalves than the freshwater mollusc species. The results also show that the omega-3 (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) fatty acids were comparatively higher in oysters, clams and cockles in marine water than those in freshwater mussels and snail. However, omega-6 fatty acids like linoleic acid (LA), α- linolenic acid (ALA) and arachidonic acid (ARA) were higher in freshwater mussels and snail than in the marine bivalves. The n-3/n-6 ratio were significantly higher in oysters and cockle species than the other groups of bivalves and snail. The index of atherogenicity and index of thrombogenicity of the mollusc species ranged from 0.74 ± 0.1 to 1.74 ± 0.2 and 0.5 ± 0.1 to 2.6 ± 0.2, respectively. The results show that marine water bivalves contained higher amount of potassium, sodium, iron, chlorine especially oyster species contained significantly higher iodine than the freshwater bivalves and snail. However, freshwater mussels and snail showed significantly higher amount of zinc contents than the marine bivalves. The heavy metal contents such as arsenic, chromium and mercury were absent or present in very tiny amounts among the mollusc species. Significantly higher amount of cholesterol was present in marine bivalves and freshwater snail species than the freshwater mussels. Overall, the results indicate that marine bivalves can be good sources of high quality protein and lipid especially EPA and DHA. On the other hand, freshwater mussels and snails also could be good sources of protein, LA and ARA but scarcity of EPA and DHA.

EXPERIMENTAL EXPOSURE OF TUNICATES *Clavelina oblonga* TO REDUCED SALINITIES AS A POSSIBLE ERADICATION MEASURE OF ITS INVASION IN MUSSEL MARICULTURE

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Introduction

In the Adriatic Sea, tunicates are a common occurrence, but invasive species *Clavelina oblonga* Herdman, 1880 (Ascidiacea, Aplousobranchia, Clavelinidae) because of its intense presence, can cause both ecological and economic losses. *C. oblonga* is a distinctive and conspicuous epi/benthic colonial tunicate naturally distributed in the Gulf of Mexico´s tropical area, on the west coast of the Atlantic, in the Azores and on the west coast of Spain (Herdman, 1880; Scarponi et al., 2018). In the Mediterranean Sea, it has been reported as invasive species (Monniot, 2001; Shenkar et al., 2022). According to the latest invasion in Northern Adriatic, beginning in the summer of 2020, the major impacts reported were associated with the bivalve industry, as aquaculture facilities with abundant suspended food and large surfaces suitable for attachment create an ideal environment for ascidians. Mussel stocks affected by biofouling can experience reduced growth, fitness and survival, with further negative impacts on shellfish farm productivity, changes in handling and defouling removal procedures, as well as the implementation of regulatory restrictions to reduce the risk of spread to unaffected areas. Considering the extent of *C. oblonga* fouling at endangered aquaculture locations, laboratory experimental exposure of *C. oblonga* to different reduced s.w. salinities (37, 30, 20 and 11) were performed during November 2020.

Materials and methods

Colonial corpuses consisting of ten to twelve *C. oblonga* zoids were placed in 8 x 5 L glass with aeration and exposed to reduced salinities of 37, 30, 20 and 11 for 14 days. Glass with s.w. salinity 37 was used as a control. Distilled water was used to achieve the wanted salinities (30, 20 and 11). Three empty, clean, complete *Mytilus galloprovincialis* shells were placed in each glass and hanged on the rope for possible *C. oblonga* larval settlement observation. After exposure, all used specimens were kept for additional 14 days in s.w. with salinity 37 for recovery treatment. Additionally, to assess the impact of treatment and to avoid feeding competition, mussels *Mytilus galloprovincialis* Lamarck, 1829 were kept separately under the same treatment as *C. oblonga* (5 L glass, aeration, feeding and salinities 37, 30, 20 and 11). Both mussels and *C. oblonga* were observed daily for filtration (valve gaping), food uptake and faeces production.

Results and Discussion

Laboratory experimental exposure of *C. oblonga* to different salinities (37, 30, 20 and 11) has shown that during the total observation time (14 days exposure + 14 days of recovery) the effects of s.w. salinity 30 did not differ in comparison to control 37, while salinities of 20 and 11 provoked disorder in *C. oblonga* feed uptake and feeding itself, leading to change of corpus colour, tissue necrosis and finally body fragmentation (Figure 1).

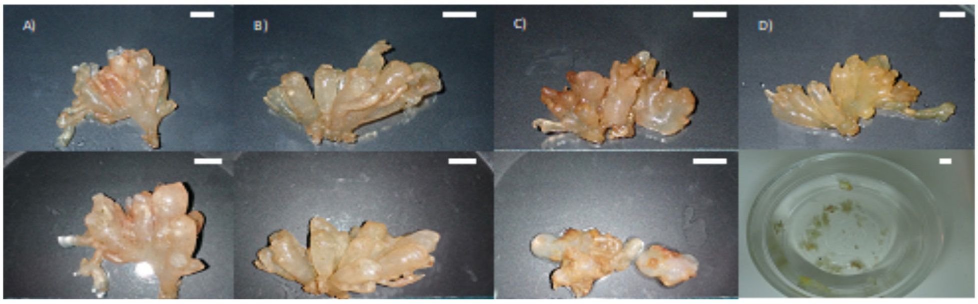

Fig. 1. Morphology of *C. oblonga* after 14 days of exposure to different salinities: A) 37, B) 30, C) 20 and D) 11 (upper row), and after 14 days recovery at 37 (lower row). The bar represents 1 cm.

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Furthermore, there was no observed egg development into lecithotrophic larva, and larvae settlement before metamorphosing into benthic adults on mussel shells or/and glass surfaces, which is in concordance with previous findings (Christianson and Eggleston, 2021). Mussels *M. galloprovincialis* kept under the same conditions as *C. oblonga* resulted in no mortality during all 14+14 days and finally, no significant fitness decrease was noticed. Only on the first day of exposure to s.w. set to salinity 11 and on the first day of the recovery period no mussel filtration activity-faeces production was observed. Based on our research, we can assume that the hypothetical translocation of mussels and whole cages with holding nets to locations of lower s.w. salinity (<20) for 7-10 days could be a natural and effective way to eradicate this invasive species. Off course, the effectiveness cannot be directly compared with the results of mussel mechanical cleaning and pressurized s.w. antifouling treatment, as well as reactive pest treatment with commercial products (Arens et al., 2011; Cahill et al., 2021), however, it can help and prevent further spreading of *C. oblonga* invasion.

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EFFECTS OF A COMBINATION OF ESSENTIAL OILS, SAPONINS AND PUNGENT SUBSTANCES ON SEA BREAM (*Sparus aurata*) and EUROPEAN SEA BASS (*Dicentrarchus labrax*) JUVENILES GROWTH PERFORMANCE AND FEED EFFICIENCY

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Introduction

Sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) are the main cultured fish species in the Mediterranean basin. Optimization of growth performance and feed efficiency has been usually done by feed formulation but the tendency to find alternatives increased. Phytogenic feed additives could contribute to those targets with a sustainable approach due to the increase of raw materials used in aquafeeds. The aim of these studies was to determine if a specific combination of essential oils, saponins and pungent substances (Syrena® Boost) can help in the continuous development of performance parameters.

Materials and methods

The first trial was run in the facilities of the Parque Científico-Tecnológico Marino (PCTM) at University of Las Palmas de Gran Canaria (Telde, Canary Islands, Spain). Sea bream juveniles of 30.7 ± 0.4 g were randomly distributed in 12 tanks of 500 L (50 fish/tank) and fed a standard diet supplemented with 2 dosages (200 & 400 mg/kg of the phytogenic combination for 12 weeks. Growth performance and feed efficiency parameters were measured along the feeding trial. Afterwards, fish were used for determination of variations in the pattern of secretion of several digestive enzymes (0, 2, 4, 8 and 24 hours post feeding (5 fish/sample, 45 fish/diet). The second trial was run in European sea bass in the facilities of the Laboratory of Applied Hydrobiology from the Agricultural University of Athens (Greece). European sea bass juveniles of 49.0 ± 0.18 g were randomly distributed in 6 tanks of 88.4 L (14 fish/tank) at a density of 7.8 kg/m^3 and fed a common commercial formulated diet supplemented with 200 mg/kg of the phytogenic combination for 20 weeks. Growth performance and feed efficiency parameters were calculated at different timepoints, and fish were sampled for somatic indexes and hematological parameters at the end of the feeding trial.

Results

Sea bream juveniles showed an improvement of FCR at both inclusion levels compared to the control diet, being better at the higher level of supplementation (1.11, 1.09 and 1.05, respectively). SGR was not affected by the inclusion of the phytogenic combination (Fig 1). Digestive enzymes patterns of secretion and response were correlated to the feed efficiency values obtained.

In European sea bass juveniles, there was a reduction of FCR in fish fed the additive of 11% compared to the control diet (1.62 ± 0.2 and 1.44 ± 0.1, respectively). SGR was significantly improved ($p<0.1$) by 13.3% as well in fish fed the diet containing the phytogenic combination (0.58 ± 0.1% and 0.66 ± 0.0 %, respectively) (Fig. 2). Final weight was also significantly ($p<0.1$) improved when fish were fed the phytogenic combination diet by 11.3% (113.1 ± 9.1g and 125.9 ± 4.5g, respectively). Blood hematocrit and hepato-somatic and spleen-somatic indices were not significantly different between treatments.

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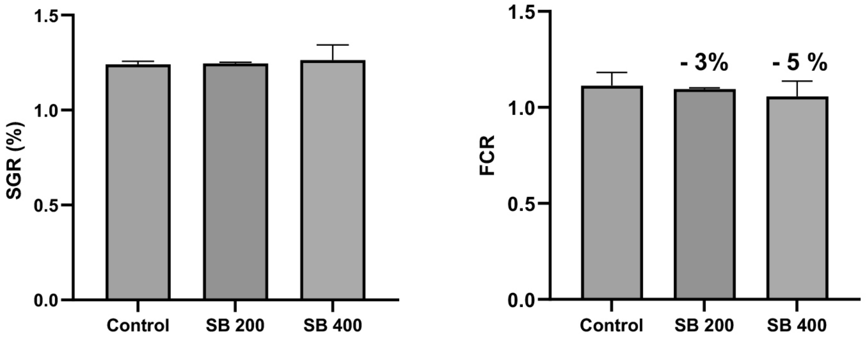

Fig. 1. SGR (%) and FCR of sea bream (*Sparus aurata*) after 12 weeks of feeding the experimental diets. Diets: 200 mg/kg (SB 200) and 400 mg/kg (SB 400) of Syrena® Boost.

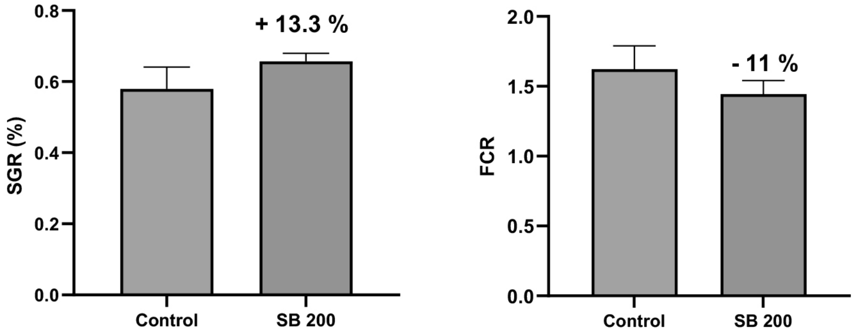

Fig. 2. SGR (%) and FCR of European sea bass (*Dicentrarchus labrax*) after 20 weeks of feeding the experimental diets. Diets: 200 mg/kg (SB 200) Syrena® Boost.

EFFECTS OF A SPECIFIC COMBINATION OF ESSENTIAL OILS, SAPONINS AND PUNGENT SUBSTANCES ON GROWTH PERFORMANCE PARAMETERS AND ANTIOXIDANT CAPACITY OF WHITELEG SHRIMP (*Penaeus vannamei*)

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Introduction

Crustacean production in 2018 reached 9.4 million tonnes, being marine shrimps dominating the production of crustaceans typically farmed in coastal aquaculture. Besides, they are an important source of earnings for a number of developing countries in Asia and Latin America. Whiteleg shrimp (*Penaeus vannamei*) is the largest produced species with 4.9 million tonnes, accounting for the 52.9% of total crusteans produced. Optimizing shrimp production performance is crucial to the success of the sector. Efficient diet formulation together with successful health and welfare management determines higher production output. As part of a proactive approach to production health and performance, the use of functional and sustainable additives, such as phytogenics, has demonstrated to be an effective tool to boost shrimp performance.

Materials and methods

Two different studies were conducted to determine the efficacy of a new phytogenic feed additive (PFA) mixture (saponins, spices and essential oils) on whiteleg shrimp growth performance and feed efficiency. In the first one, six hundred SPF shrimps of average initial weight of 1.9±0.26 g were allocated into three groups using with four replications for each treatment in 350 L tanks. In the second one, two hundred and forty shrimps of average initial weight of 1±0.01 g were randomly allocated into three groups with four replications for each treatment in 20 L baskets. In both studies, shrimps were fed for 6 weeks the different treatments: one control group (basal diet) and two groups receiving Syrena® Boost included at 200 & 400 mg/kg of feed, respectively.

Results

After 6 weeks of feeding, results in the first study showed that shrimp survival did not differ between treatments being above 80%. In terms of growth performance, shrimps fed Syrena® Boost at both inclusion rates showed an increase of weight gain of 12.5% and 13.3%, respectively, with an increase in average daily growth (ADG) of 12.9% and 13.5%, respectively. Feed conversion ratio (FCR) was similar between treatments. In the second study, again shrimp survival did not differ between treatments being above 94% in all treatments. Shrimps fed Syrena® Boost at both inclusion rates showed again an increase of weight gain of 10.1% and 7.9%, respectively, being statistically significant ($p \leq 0.1$) at the lowest dose. ADG was also improved by 9.7% and 7.9%, respectively, again being statistically significant ($p \leq 0.1$) at the lowest dose. FCR was reduced by 3.7% and 1.7%, respectively. Altogether, these results indicate the positive growth performance effects of this new PFA in whiteleg shrimp.

ANTIMICROBIAL ACTIVITY IN MICROALGAE: A WAY TO IMPROVE SUSTAINABILITY IN AQUACULTURE

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Introduction

High population densities and stress in intensive fish farming may lead to problems with diseases, which result in the use of antibiotics. The development of resistant strains in fish farms makes the use of antibiotics inefficient. At the same time, a negative image of the aquaculture industry among consumers is formed. (Martinez et al., 2012). In the present study, antimicrobial activity against three fish pathogenic bacteria *Vibrio anguillarum*, *Aeromonas veronii*, and *Vibrio alginolyticus* was determined in cultures of seven microalgae species (*Amphidinium carterae*, *Asteromonas gracilis*, *Tetraselmis* sp. (red var.), *Tetraselmis* sp. (Pappas), *Tetraselmis* sp. (red var., Kotichi), *Tetraselmis* sp. (palmella), and *Tetraselmis marina* (var. Messolonghi), isolated from lagoons in Western Greece.

Materials and methods

Bacterium-free microalgae cultures were obtained after treatment with a mixture of antibiotics (oxolinic acid 10 µg/mL, kanamycin 10 µg/mL, penicillin G 150 µg/mL, streptomycin 75 µg/mL) (Munro et al., 1995), to kill bacteria present in the cultures. The absence of live bacteria was verified by plating on tryptic soy agar (TSA) dishes added 2% NaCl (w/v). The antibiotic was removed after successive dilutions over 3-4 weeks. *Chlorella minutissima* was used as a reference species, as it has shown antibacterial properties in an earlier study (Kokou et al., 2011). After algae cultures reached the late exponential phase, aliquots of 5 mL of each of the microalgae cultures were inoculated with different bacterial pathogens in test tubes. The pathogenic bacteria were added at a final concentration of 10^4 CFU/mL. The growth of the added pathogens was followed 0, 1, 2, 4, and 6 days after inoculations by spreading 10-fold dilutions on TSA dishes, and the colonies were counted after incubation for 7 days (Kokou et al., 2011). A positive control treatment was run for each pathogen, where pathogenic bacteria were added in 25 ppt sterile seawater, enriched with Walne's medium. The experiments were performed both in the presence and absence of light.

Results and Discussion

In all microalgae cultures, the numbers of bacteria were lower compared with the positive control treatments, where the numbers of bacteria increased exponentially. In general, antimicrobial activity was higher in light, especially in the case of *A. gracilis*. Weaker antimicrobial activity was observed in cultures of *Tetraselmis* sp. (palmella), *Tetraselmis* sp. (red var., Kotichi) and *Tetraselmis* sp. (red var., Pappas). In cultures of *A. carterae*, no difference between light and dark was observed. Its antimicrobial activity was lower compared with the other cultures, except in the case of *A. veronii* where it proved to be the most effective. Among *Tetraselmis* isolates, the highest antimicrobial activity was detected in *Tetraselmis* sp. (red var). Cultures of *A. carterae*, *Tetraselmis* sp. (red var.) and *A. gracilis* reduced dramatically the number of cells of *A. veronii* after 7 days of observation, as shown in Figure 1. In the case of *A. gracilis* and *Tetraselmis* sp. (red var.), the numbers of pathogenic bacteria decreased dramatically on the second day of the experiment, while in *A. carterae* this occurred on the fourth day of the experiment.

Acknowledgements

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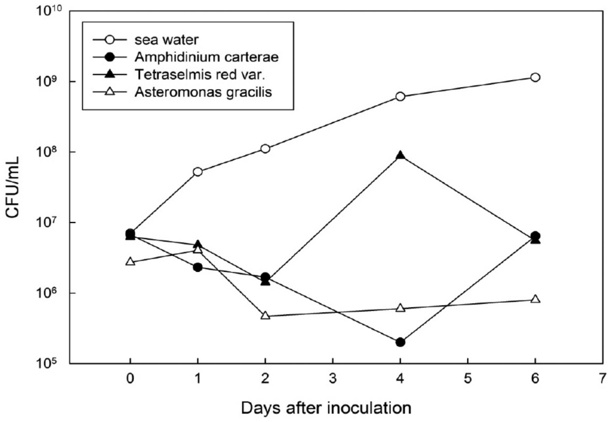

Fig. 1. Colony-forming units/mL (in log scale) of *A. veronii* in experiments performed with cultures of *A. carterae*, *Tetraselmis* sp. (red var.) and *A. gracilis* compared with sterile seawater 25 ppt, through time in light conditions.

- The strongest antimicrobial activity against *V. anguillarum* was shown by the cultures of *Tetraselmis* sp. (red var., Pappas) and *Asteromonas gracilis* both in dark and light conditions.
- In the presence of light, the cultures of *A. carterae*, *Tetraselmis* sp. (red var.) and *A. gracilis* were the most efficient against *A. veronii*, and in the dark, the cultures of *Tetraselmis* sp. (red var.) and *A. gracilis*.
- Against *V. alginolyticus*, in light conditions, the cultures of *A. carterae*, *Tetraselmis* sp. (red var.) and *A. gracilis* were the most effective, and in the dark the cultures of *A. gracilis*.

EFFECT OF OXYGEN SATURATION ON EXPRESSION OF *TRYPSIN*, *PLA2,* AND *HSL* IN EUROPEAN SEA BASS (*Dicentrarchus labrax* L.)

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Introduction

In commercial fish farming, the oxygen dissolved in water (DO) and water temperature are the main environmental parameters that directly affect the physiological functions of the cultured fish. In summer and early autumn, low DO levels will cause the fish to become stressed and may decrease appetite and feed/energy utilization, ultimately leading to lower growth (Vanderplancke *et al*., 2015), which again reduces productivity in fish farming. In this study, we investigated the effects of three different DO saturation ranges (40-60%, 60-80%, and 80-100%) on the expression of three key enzymes involved in lipid and protein metabolism, namely phospholipase (PLA2), hormone-sensitive lipase (HSL), and trypsin in European sea bass (*Dicentrarchus labrax* L.).

Materials & Methods

The effect of DO saturation was studied in an experimental recirculating land-based system, at HCMR, Crete. Seabass juveniles with an initial weight of 88 g were stocked in 500 L tanks (35 fish per tank). Three treatments with different DO saturation ranges of 40-60%, 60-80%, and 80-100% were used with three replicate tanks per treatment for 98 days. The weight of fish populations was measured at the beginning of rearing conditions, as well as at the end, when tissue samples were isolated from the anterior and distal intestine (AI and PI), liver (L) and pyloric ceca (PC) of the fish. Expression of each gene was studied in six samples per tissue and tank. After total RNA extraction, a two-step qRT-PCR was performed, and the relative transcript abundance of each gene was normalized to *L17a* expression. All primers were taken from existing literature. Analysis of qPCR results was carried out using the $2^{-\Delta\Delta Ct}$ method. Statistical significance of differences in gene expression between treatments was tested with ANOVA.

Results and Discussion

At the end of the experiment, the average weight at 40-60% DO saturation (197.2±0.8 g) was significantly lower compared with 80-100% (252.4±4.5 g) and 60-80% (230.7±7.3 g). *PLA2* was expressed in PC, AI, and DI samples, while no expression was detected in the liver. In contrast, *HSL* expression was limited to the liver, while expression of *trypsin* was detected in all four tissues. Expression of *trypsin* and *PLA2* was found significantly lower at 80-100%, compared with the other two treatments ($p<0.05$), while expression of HSL was found significantly higher at 60-80% and 80-100% DO saturation ranges, compared with 40-60% ($p<0.05$). A similar expression pattern to *trypsin* was found in AI samples, and similar pattern to *PLA2* in AI and DI samples, but this was not verified statistically ($p>0.05$). Although low DO conditions are usually linked to low digestive enzyme activity (and low gene expression), several studies suggested that under moderate hypoxia, the organism turns to a "digestion-priority mode" (Zhang *et al*., 2010). This may explain the higher expression levels of *trypsin* and *PLA2* in the lower DO ranges, in our study. *HSL* is activated during periods of energy demand when the organism needs to mobilize energy stores. Higher expression of *HSL* at high DO saturation levels may indicate this mobilization of energy stores, associated with an increased metabolic rate, a possible result of a long-term acclimation to an increased oxygen uptake. Further analysis, including digestive enzyme activity, is required to explain the underlying mechanisms by which DO variations affect the physiology and performance of fish.

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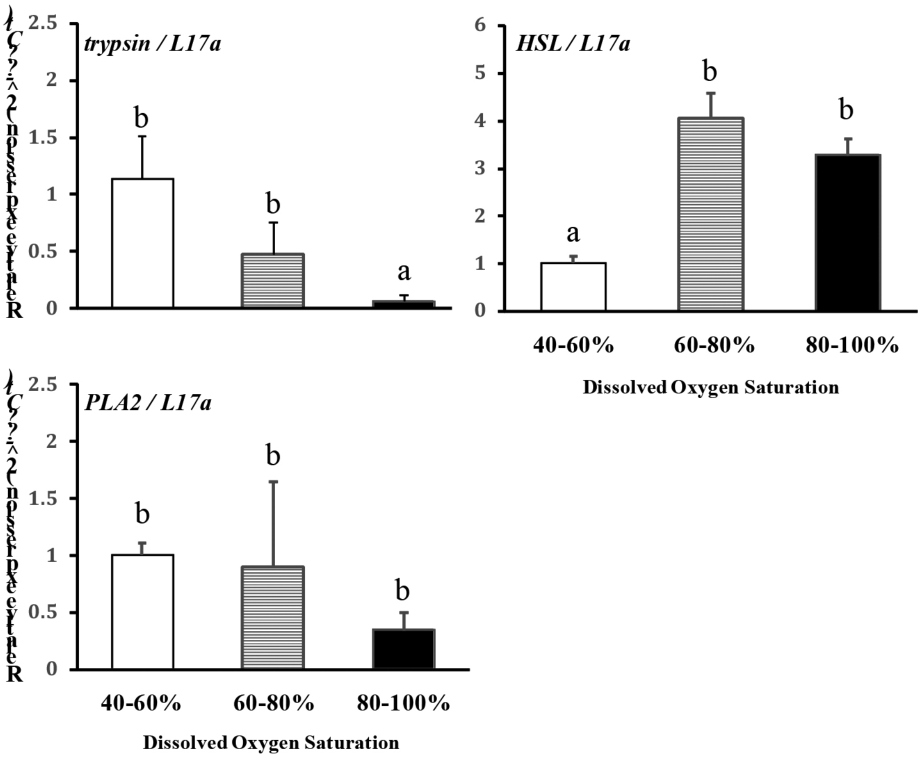

Fig. 1. Relative expression (mean±SE n=3) of *trypsin* and *PLA2* as quantified in pyloric ceca) and *HSL* in the liver, under the effect of different DO saturation levels in seabass. The numbers in brackets indicate the number of samples used for the analysis. Different letters indicate statistically significant differences between groups (p<0.05).

In conclusion:

- expression of trypsin and PLA2 was significantly lower at 80-100% DO compared with other two treatments, while
- expression of HSL was significantly higher at 60-80% and 80-100% compared with 40-60% DO saturation range.

Acknowledgments

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DEVELOPMENT OF A HISTOLOGY-BASED HEALTH ASSESMENT PROTOCOL FOR FISH REARED IN RASs

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Introduction

Recirculating aquaculture systems (RASs) show several advantages compared with flow-through systems, such as year-round production, reduced ecological footprint, reduced costs for temperature regulation, and increased biosecurity (Badiola *et al*. 2012). Despite the long-term use of RAS technology (Campanati et al., 2021), there is still a lack of monitoring tools - and therefore a lack of control - over the parameters related to fish health and, with various biotic and abiotic factors affecting the health status and thus the quality of the fish. The effect of such factors can be monitored through histological analysis of specific organs (Saraiva et al. 2015) even well before affecting the overall health status of the fish. The objective of the present study was to monitor and assess the health status of seabass and seabream, reared in RASs (Andromeda facilities, Western Greece), using a qualitative histological analysis.

Materials & Methods

Two seabass (*Dicentrarchus labrax*) and two seabream (*Sparus aurata*) populations reared at Andromeda facilities were monitored in different RAS for each fish species. Four samples were taken from the seabream populations, from March to June 2021 (54-167 dph, 0.07-3.19 g average weight), and three samples were taken from the seabass from March to April 2021 (91-142 dph, 1.07-5.05 g average weight). Five to ten fish or isolated organs (liver, spleen, intestine, and gills) were fixed in 4% formaldehyde for histological analysis. Three fish were selected from each sampling point and pieces of tissue from targeted organs were embedded in Technovit 7100 (Heraeus Kulzer, Germany), sectioned at 5 μm thickness and stained with Polychrome I / Polychrome II. Ten sections per targeted tissue of each fish were examined under a light microscope (Observer Z1, Zeiss, Germany) and photos were taken at 10×, 20×, and 40× magnification. Histological changes were assessed qualitatively by noting the presence or absence of specific deviation from healthy tissues.

Results and Discussion

The preliminary results of this study showed only slight deviations from healthy tissues in the gills, liver, spleen, and intestine of both fish species. No cause-specific mortality and no disease outbreaks were observed during the sampling period, indicating an overall good health of the monitored fish. Deviation from healthy tissues, as determined by histological observations included: uplifting of the epithelium of secondary lamellae was observed in gills, as well as a basal fusion of secondary lamellae (Fig.1, top left), an abnormality related with parasite infestation in various fish species. The histological analysis showed a healthy or slightly modified liver with vacuoles consisting of dead cells and neutrophil liquid in some cases (Fig.1, top right), or an accumulation of glycogen. Histology of the intestine was normal or presented slight aggregation of myxospores in rare cases (Fig.1, bottom right). Finally, histological analysis revealed multifocal lipid accumulation in the spleen, in rare cases (Fig.1, bottom left). Multifocal melanomacrophage centers were also observed in the spleen and liver.

Fish histology is a tool to evaluate rearing conditions in rearing system and is widely used as a health assessment tool. In our study, we used descriptive histology to make a preliminary evaluation of the health status of seabass and seabream reared in RASs. The final aim was to quantify histological alterations observed in targeted organs of fish reared in RASs and develop a histology-based quantitative health assessment protocol through which monitoring of the health status of fish and – on a second level – of the parameters that prevail in RASs will be possible.

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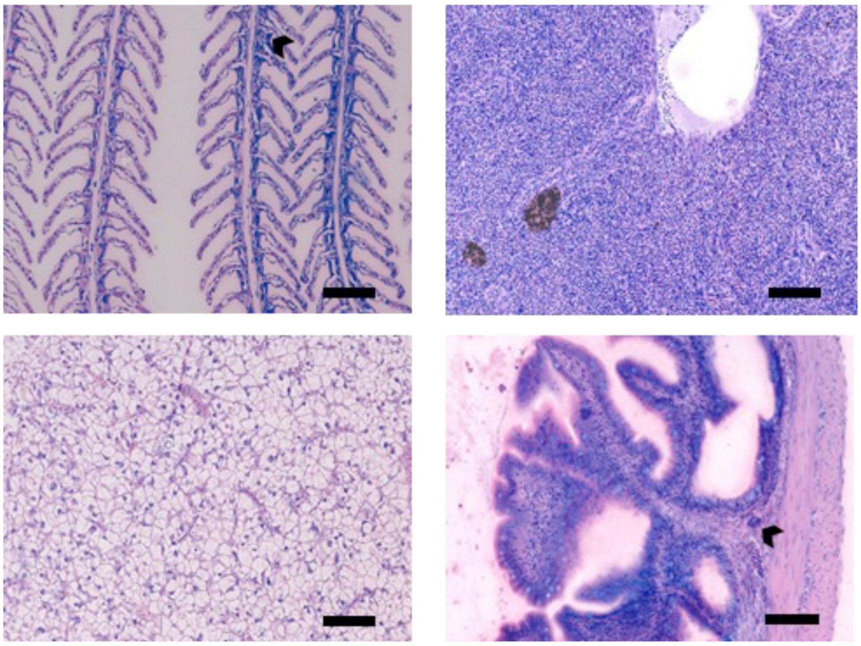

Fig. 1: Basal fusion of secondary lamellae (arrowhead) in gills (top left). Space consisted of dead cells and neutrophil liquid in the liver (top right). Multifocal lipid accumulation in the spleen (bottom left). Aggregation of myxospores (arrowhead) in the intestine (bottom right). 20× magnification and bar 100 µm.

Acknowledgements

This work was supported by the research project “Optimizing land-based fish production in next generation digital recirculating aquaculture systems” which was funded by General Secretariat for Research and Innovation and EU funds.

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PRELIMINARY RESULTS ON THE INCLUSION OF LYOPHILIZED YEAST *Rhodotorula mucilaginosa* IN GILTHEAD SEA BREAM *Sparus aurata* DIET

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Introduction

The rapid development of global aquaculture sector led the research interest in issues concerning the nutrition of intensively reared fish. The establishment of efficient feeding protocols is essential for optimizing fish growth and improving feed conversion rates, as food must be available in sufficient quantities and with appropriate composition to maintain fish health. The use of functional feed additives has been increased the interest of the aquaculture sector, since the improvement of the fish feeds' quality and fish health is increasingly becoming an important topic for producers. Yeasts and their bioactive compounds can enhance fish growth, immunity, and performance (Agboola *et al*., 2021). The yeast *Rhodotorula mucilaginosa,* that can be found in the gut microbiota of wild fish species, could be a useful and promising additive with growth and immune boosting properties (Wang *et al*., 2019; Chen *et al*., 2019). The aim of this study was to evaluate the effects of fish feed containing three different inclusion levels of lyophilized *R. mucilaginosa*, on growth of the gilthead seabream *Sparus aurata*, a species of great economic importance for the Mediterranean aquaculture.

Materials and methods

Four experimental isolipidic, isoproteic and isoenergetic pelleted diets containing lyophilized *Rhodotorula mucilaginosa* ACA-DC 5340 at the inclusion levels of 0%, 1%, 2% and 3% (C, R1, R2 and R3, respectively), were formulated at the Aquaculture Laboratory of the University of Thessaly. The yeast was obtained by the ACA-DC Collection of the Agricultural University of Athens, grown on Potato Dextrose Agar (PDA) plates and lyophilized into a dry powder. The experimental diets were performed with a laboratory California Pellet Mill (CL-2, Irmeco GmbH, Netherlands). Gilthead seabream individuals were obtained from a commercial fish hatchery (Philosofish S.A.) and transferred to the experimental fish facilities at the Laboratory of Applied Hydrobiology of the Agricultural University of Athens. One hundred twenty fish were acclimated to laboratory conditions, under a controlled photoperiod (12h:12 h light/dark) and were randomly distributed into 12 tanks (174L). Fish with a mean weight of 199,8±1,56g were classified into four groups and each group included three replicates. Fish were fed, by hand twice daily, at a rate of 1,2% BW. During the whole experimental period, water quality parameters were monitored routinely with water temperature being maintained at 20.7 ± 0.07°C, pH at 7.4±0.03 dissolved oxygen at 6.6±0.5mg/L. Fish were individually weighed on a regular basis and food quantity was adjusted accordingly. Growth performance was determined and feed utilization was calculated as follows:
Weight gain rate (WGR, %) = 100 × (final body weight-initial body weight)/initial body weight.

Fulton's condition factor K (gr/cm3) = 100 × final weight × fork length^{-3}
SGR (% day^{-1}) = 100 × (ln final body weight - ln initial body weight) × days^{-1}
FCR = Feed consumed × Body weight gain^{-1}
TGC = 1000 × (Final body weight$^{1/3}$- Initial body weight$^{1/3}$) × temperature degree days^{-1}

Results

The total weight gain did not differ significantly between the four groups. The R1 group, exhibited the highest weight gain rate. No differences were observed for the final weights among groups. The SGR and FCR ratios did not differ significantly. However, R1 group exhibited a higher SGR and a lower FCR ratio compared to the C, R2 and R3 groups. On the other hand, R3 group recorded the lowest feed consumption. TCG was also higher for group R1. Finally, although the condition factor did not differ significantly, the control group exhibited a slightly higher condition factor than groups fed diets with *R. mucilaginosa* inclusion.

Discussion

Yeasts are promising ingredients that can be used as supplements in feeds due to their relatively high protein, energy, and micronutrient content (Glencross *et al*., 2020). In the present study, sea bream fed diets with 1% inclusion of *Rhodotorula mucilaginosa* exhibited better somatic indices. Similar results were observed in Nile tilapia (*Oreochromis niloticus*) fed diets with 1% of the same yeast in a hydrolyzed form (Chen *et al.,* 2019). Nevertheless, it has been shown that higher dietary levels of yeast may result in reduced palatability, feed intake and growth (Gamboa-Delgado & Marquez-Reyes, 2016). This may explain the lower feed consumption in the experimental group fed 3% of yeast. Further investigation is necessary to assess the different levels of *R. mucilaginosa* inclusion and the benefits that could have on the overall fish health and performance.

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THE PARTNER SELECTION AND SPAWNING BEHAVIOR IN PIKEPERCH (*Sander lucioperca*) DURING SEASONAL NEST SPAWNING

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Introduction

The pikeperch is valuable for aquaculture production due to its flesh quality and high market value (Blecha et al. 2015; Nebeský et al. 2016). However, more than 95% of the market supply has been provided by capture (FAO, 2018; Policar et al. 2013; Steenfeldt et al. 2015), leading to the decline of wild populations in central, eastern, and northern Europe (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..

One of the concerns in pikeperch populations dynamics is the disproportion of the sex ratio, where females dominate in a size group above 550 mm TL (total length) (Raikova-Petrova and Živkov 1998; Gröger et al. 2007; M'Hetli et al. 2011) Stizostedion lucioperca (L.. Gillnet fishing has the most significant impact on the disproportion, as the most prominent individuals are usually among fisheries catches (Gröger et al. 2007; Vainikka and Hyvärinen 2012)biomass B, recruitment R, spawning stock biomass SSB, yield-per-recruit Y/R, catch-to-biomass ratios Y/B. Such imbalances occurring within the wild population of pikeperch could favour a decrease in reproduction efficiency. It can drastically affect the amount of energy the population invests in reproduction since larger females contribute disproportionally more to population replenishment (Barneche et al. 2018; Olin et al. 2018); therefore, the most productive specimens pair with an unequally sized partner.

This study aimed to investigate the preference of male size by the female during natural spawning on the nest. We hypothesise that variations in spawning behaviour related to the different sizes of partners could influence the reproduction process and the success of pikeperch.

Material and methods

In late October, adult pikeperch individuals were collected from the production ponds of the Nove Hrady Ltd. and transferred to a small earthen pond (0.25 ha) for overwintering. At the beginning of April, females with oocytes in stage V (BW = 920 ± 69; Bodyweight) and males of two sizes: large (BW = 1388 ± 163 g) and small (BW = 566.7 ± 66.9 g); released milt, were selected for spawning in the nests. A set of fish consisting of one female and two different sized males were transferred to an outdoor flow-through aquaculture spawning chamber: Length: 2.8m; Width: 1.5m; Height: 0.8m; total volume of 5.6 m^3) with two artificial spawning nests (0.9 m in diameter) made of stiff long hair brush (Malinovskyi et al. 2018). The water temperature was measured at 1-hour intervals using Minikin Tie digital thermometer (Enviromental Measuring System s.r.o., Czech Republic) placed inside the inflow water pipe. Nests in the spawning chamber were checked twice a day with a 12-hour interval until the female had paired with one of the males. Male size and occurrence of the spawning were recorded. All nests were photographed, and photos were processed using QuickPHOTO MICRO 3.0 software. The empty nests processing included calculation of: (I) cleaned nest area, % of total nest area; (II) cleaned area centring, mm from the centre of the nest. For the nest with egg processing, they further included (III) calculation of egg distribution area, % of total nest area; (IV) clustered eggs area, % of total nest area; (V) clustered eggs area, % of the distribution area. Subsequently, the nests with eggs were transported to the RAS system for further incubation under controlled conditions.

After each successful spawning, blood samples from all fish were collected to determine testosterone for males and estradiol for females in the blood plasma. The testosterone and estradiol levels in the blood plasma were measured by immunoanalytical ECLIA methods (Cobas 6000, Roche, Manheim, Germany).

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Results and discussion

Observed behavioural patterns have indicated that the differences in cleaning efforts of pikeperch males were recognised by females and determined the selection of the partner. Generally, pikeperch females have preferred bigger males in 77% of recorded spawning events. However, statistical analysis has indicated that the mechanism of selection was not related to the bodyweight of the male but rather to its efforts in nest preparation. All big males have exhibited cleaning efforts toward the nest, compared to just 50% among small males, confirming the suggestion of better consistency and efficiency of spawning behaviour expression in larger fish. Despite appropriate body weight and cleaning efforts, 33% of the big males were not selected for spawning due to their inability to efficiently recognise and utilise the nest area. The preferred small males were significantly more precise in identifying and using the nest area compared to unpreferred big and small fish. This could be crucial for expressing paternal care and an explanation for females' selection.

The statistical model indicated the male size, plasma testosterone, and average daily temperature significantly affected the parameters of cleaned nest area, % and cleaned nest area centring, mm (Table 1). Higher testosterone values were associated with the larger cleaned size of the nest and its more precise placement relative to the total nest area, e. g., the lower distance between centres of the areas. Similarly, a lower temperature (range: 11.3 – 12.5 °C) was associated with a better cleaning performance of pikeperch males, regardless of the size (Table 1; Figure 1). Therefore, females preferred males cleaning larger nest areas and more precise placements on the nest.

The statistical analysis has also indicated that the water temperature significantly influenced the testosterone levels of males (Figure 1). Females paired with big males showed an insignificant tendency of higher estradiol levels than those selected small males. In addition, testosterone in male blood plasma had increased within a temperature range of 11.6 - 12.5 °, which explains the better cleaning performance of males in this temperature range.

There were no significant effects of the factors mentioned above on parameters of egg distribution, egg clustering and larvae production (Table 1). These characteristics were stable through the experiment, suggesting that selection availability allowed females to exhibit consistent spawning behaviour.

To the best of our knowledge, this is the first study on pikeperch describing a relation between water temperature and testosterone levels in the blood plasma of males. This study confirms the importance of male spawning behaviour in the natural reproduction of pikeperch. Such data could be valuable in predicting the reproduction output in wild pikeperch populations and may guide the controlled reproduction of wild and pond-cultured pikeperch broodstock.

Acknowledgements

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TOWARDS AN OPTIMUM MICROBIOME COMPOSITION FOR CULTIVATION OF THE SEAWEED *Ulva ohnoi*: RESULTS FROM THE SIMBA PROJECT

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Introduction

The interest in micro- and macroalgal biomass for use in human and animal food and feed products, newly developed applications as seaweed carbohydrates for biodegradable plastics packagings among others is growing exponentially worldwide. Large-scale cultivation is required to meet this growing demand of seaweed biomass. Although it has been known for a very long time that the seaweed microbiome is essential for their healthy growth and development (e.g. Provasoli & Pintner, 1980), many questions are still open considering the mechanism to which the microbiome influences growth and composition and how this is influenced by cultivation conditions (temperature, light, nutrients, etc.).

Within the framework of the SIMBA project (Sustainable innovation of microbiome applications in food systems), growth and chemical composition of the chlorophyte seaweed species *Ulva ohnoi* and the composition of its microbiome has been studied. Causal relationships will provide links between microbiome and algal morphogenesis, growth and nutritious values will be studied. The final ambition of this activity of the SIMBA project is to formulate the optimal microbiome composition for sustainable seaweed production (Ulva) in large outdoor tanks.

Material and methods

Ulva ohnoi M.Hiraoka & S.Shimada, collected from a former salt evaporation pond in the south of Spain, was grown in three different types of cultivation systems with an increasing degree of control on the growth conditions (Fig. 1).

- System 1 (EP): cultivation in floating cages in its natural environment (former salt evaporation ponds), renewal of natural seawater by tidal movements.
- System 2 (OT): cultivation of seaweed biomass in outdoor 400 L tanks. Weekly medium change with nutrient enriched treated natural seawater.
- System 3 (PBR): cultivation in 80 L fiberglass photobioreactors in cultivation chamber (constant temperature and controlled illumination). Weekly medium change with nutrient enriched, filtered and treated natural seawater.

Relative growth rates of the seaweeds were calculated monthly to bimonthly in the EP systems and weekly in the other two systems as weight increase assuming exponential growth. Every month, biomass was harvested from all three cultivation systems, seaweed thalli were gently rinsed in sterilized artificial seawater and spread in petri dishes in a flow cabinet. Samples of the seaweed microbiome were obtained by swiping sterile cotton swabs over the thallus (Fig. 2), after which they were stored at -80 °C until analyses. To be able to discriminate between the seaweed microbiome and the water microbiome, 2 L water samples were taken on each sampling event and filtered through a series of sterile filters (20, 5 and 0,45 µm); microbiomes were finally collected in 0.22 µm Sterivex filters. Additional biomass samples were frozen for later determination of proximate composition. Microbiome composition of the seaweeds and water samples was determined through ribosomal RNA (partial 16S rRNA gene) amplicon sequencing.

Results and discussion

Growth in EP and OT peaked in June and July and was lowest in winter and early spring. In general, growth in OT and EP appeared to be mainly driven by irradiance and water temperature. Episodes of high salinity (50 psu) in EP might have had a negative effect on growth rates in late summer. In EP biomass disappeared completely in December and January, whereas in OT positive growth rates were recorded whole year long, albeit low (< 1% d^{-1}) in winter. Growth in PBRs was generally quite constant (8-10 % d^{-1}), although sudden collapses ocurred, characterised by thallus degradation and negative growth rates. The latter was also observed two times in the outdoor tanks. In one occasion, this collapse was probably related to an outbreak of the parasitic brown macroalga of the *Myrionema* genus both in the PBR and OT systems. Proximate composition of the seaweed biomass was constant in the PBR systems but showed seasonal variation in both outdoor systems.

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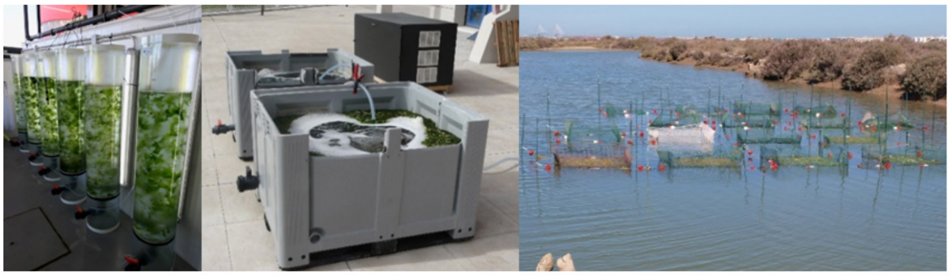

Fig. 1: Cultivation systems for the chlorophyte *Ulva ohnoi;* indoor photobioreactors (left), outdoor tanks (centre), outdoor floating cages (right).

Fig. 2: Seaweed thallus microbiome sampling using sterile cotton swabs.

Laboratory studies have shown that the seaweed chemosphere, related to its microbiome can affect growth rates and possibly chemical composition (Wichard 2015; Ghaderiardakani et al. 2020). In this study, microbiome composition on the seaweed thalli will be compared both within system in time and between systems, focusing on the periods of maximum growth and the collapses. This way, the intention is to identify the bacterial species that might have a positive effect on seaweed growth and form the basis of an optimal microbiome inoculum

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TOWARDS A FREE WILD FISH AND SOY DIET FOR EUROPEAN SEABASS USING BY-PRODUCTS FROM FISHERY AND AQUACULTURE

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Introduction

The rapid development of aquaculture, in last decade, has made this sector one of the most important both at economic and social level gaining a main role in human nutrition, but this industry is severely limited by the lack of proteins intended for animal feed (as a competitor of human nutrition) (Wang et al. 2015). Fishery and aquaculture by-products can be considered as promising alternative feed ingredients in terms of nutritional quality and availability; however, these products are still underused resulting in economic and environmental issues (Gasco et al. 2020)that is, insect meal and fish by-products, as alternatives to conventional animal protein sources. After an introductory part that highlights the need for sustainable development of animal production, the alternative protein sources are discussed. In particular, after providing some indications on their production and supply focussing on EU, a SWOT (Strengths, Weaknesses, Opportunities, and Threats. At the same time, limiting the use of soy in fish diets has become necessary for the sustainability of aquaculture production. In past years, due to emerging health concern, gluten meal has become an even more significant cereal by-product of agriculture that is spreading thanks to its high protein content (Tapia-Hernández et al. 2019). The effects of total replacement of wild fish meal, (FM) fishoil (FO) and soy product (SP) by using fishery and aquaculture by-products and gluten were tested on the growth, gut health and fish quality parameters of European seabass.

Materials and Method

Five experimental diets (control C, 0FM100FO, 0FMFO, 0FMFO-50SP, 0FMFO-0SP) were formulated to totally replace wild fishmeal (FM), wild fish oil (FO) and soy protein using fisheries and aquaculture by-product, and gluten protein. 50 Sea bass individuals (initial weight: 75.96±6.99g) were reared in recirculated aquaculture system for 119 days, during the on-growing phase. Temperature was maintained constant at 22±0.5°, photoperiod was set on 12h day length through artificial light and water parameters, as salinity, dissolved oxygen and nitrogen compound were daily monitored. At the end of the trial, growth indexes, feed intake (FI), proximate composition, somatometric indexes, blood plasma biochemistry were detected. The assessment of gut microbiota (GM) was made by Next-generation sequencing. The assignment of all major NMR signals of the perchloric extracts was performed and a multivariate classification analysis was applied on the entire dataset to reveal metabolites important for characterizing samples according to the diets. Differences among treatments were considered significant at $P < 0.05$.

Results

Final body weight was significantly higher in diet C and 0FM100FO compared to other treatments. Specific growth rate and weigh gain were higher in C compared to 0FM100FO. 0FM0FO-50SP was lower than 0FM100FO, but meantime, 0FM0FO and 0FM0FO-0SP were lower compared to other treatments. FI values were significantly higher in 0FM0FO then 0FM0FO-0SP. FCR, was statistically lower in C compared to 0FM0FO, 0FM0FO-50SP and 0FM0FO-0SP. Values of proteins in 0FM100FO were significantly higher compared to 0FM0FO. Protein efficiency ratio was statistically higher in C then 0FM0FO, 0FM0FO-50SP and 0FM0FO-0SP, while 0FM0F0-0SP was higher than 0FM0FO and 0FM0FO-50SP. Gross protein efficiency was lower in 0FM0FO and 0FM0FO-50SP compared to C and 0FM100FO. Results of viscerosomatic index were statistically lower in diet C compared to 0FM0FO-0SP. 0FM0FO-0SP value of hepatosomatic index was higher compared to 0FM0FO-50SP, 0FM0FO and C, at the same time 0FM0FO-50SP was lower than 0FM0FO-0SP. Results of Lipid efficiency rate and inorganic phosphorus shown a P-value lower than 0.05 but not specific difference among treatments was evaluated by multiple comparison Tukey's test. In animal fed with C diet, creatine value was higher confronted to other treatments. Results of Uric acid shown higher value in diet C confronted to 0FM0FO, 0FM0FO-50SP

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and 0FM0FO-0SP. Alkaline phosphatase and Total protein were both lower in diet 0FM100FO compared to 0FM0FO-0SP. Magnesium shown higher value in diet C compared to 0FM0FO-0SP. High density lipoprotein value was lower in 0FM0FO compared to 0FM0FO-0SP. Albumine/Globulin was higher in 0FM0FO compared to 0FM0FO-0SP. Values of Lactate were lower in OFM100FO, 0FM0FO-50SP and 0FM0FO-0SP compared to other treatments.

Discussion

This study highlighted the possibility to total replace wild FM and FO using by-product from fisheries and aquaculture with only a marginal reduction of the overall performance and considering the positive implication on the economic and environmental impact at industrial level. In particular, when only wild FM was totally replaced by fisheries by-product no differences were recorded, while the combine replacement of wild FM and FO resulted in a performance reduction. Interestingly, the further replacement of soy products by alternative plant proteins in the free wild fish diet did not result in a decline of performance. As the fish quality is a broad and complex concept embracing many components, the metabolomics study applied in this work will provide a comprehensive descriptor consisting of a pattern of molecular components undergoing metabolic changes related to the different diets.

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NATURE´S CONTRIBUTIONS TO PEOPLE PROVIDED BY LOW-TROPHIC SPECIES AQUACULTURE

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Introduction

AquaVitae (AV) is a research and innovation project funded by the EU's Horizon 2020 program. AV's overall objective is to introduce new, and expand existing, low trophic species (LTS) products and processes to marine aquaculture value chains across the Atlantic. The value chains that AV focuses on include macroalgae, integrated multi-trophic aquaculture, echinoderms, underutilized shellfish species and low trophic finfish species. Moreover, AV includes analysis of value chains, market development and profitability, and other biological and socioeconomic aspects, including sustainability, environmental monitoring, and risk assessment of LTS aquaculture.

The achievement of sustainable aquaculture requires consideration of the positive and negative impacts of aquaculture on the environment, economy, and society. The identification and quantification of the ecosystem services (ES) derived from LTS aquaculture can provide useful information to support a rigorous sustainability assessment of the three sustainability dimensions (environmental, economic, and social). Nature's Contributions to People (NCP) is a novel framework for ES, defined as all the contributions (positive and negative) of nature to people's quality of life, and include the role that humans play in the co-production of benefits from nature. This framekork fits well the scope of AV and was therefore selected for the quantification of ES as part of the sustainability analysis of LTS aquaculture in the Atlantic Region. The present work aims to identify and quantify the NCPs provided by LTS aquaculture, based on the case studies (CS) investigated within AV. Additionally, it identifies knowledge gaps in terms of what NCPs are supported by LTS aquaculture, and what data were missing for quantification of the services provided.

Material and methods

The work was deconstructed into four sub-tasks: i) identification of the NCPs provided by LTS (based on the CS investigated within AV) and identification of current knowledge gaps in terms of qualitative contributions of LTS aquaculture to the NCPs using expert judgements and literature; ii) selection of specific CS to be included in the evaluation of NCPs provided by LTS aquaculture based on the number of identified NCPs and overall knowledge level (estimated based on the data quality scoring for the identified NCPs in the previous step); iii) selection of indicators for NCPs quantification (based on the framework developed in a previous task from AV); and iv) quantification and analysis of selected NCPs using the most appropriate valuation method for each indicator using data from CSs within AV and literature reviews.

Results and Discussion

Overall, a relatively high number of NCPs were identified and confirmed for all CSs assessed. The highest number of identified NCPs was reported for CS4 *Sea-based IMTA* and CS9 *Mussels*, both with 15 identified NCPs out of 18, followed by CS2 *Offshore macroalgae* and CS8 *Oysters* both with 14 identified NCPs, and two land-based systems, CS1 *New macroalgae species* (13 NCPs) and CS3 *Land-based IMTA* (11 NCPs). The lowest number of identified NCPs was reported for CS6 *Sea urchins* with 9 identified NCPs, which may be explained by the immaturity of this industry compared to other more established LTS aquaculture industries. Based on defined selection criteria, the CSs *Offshore kelp production*, *Land-based IMTA*, *Oysters*, and *Mussels* were selected for the subsequent NCP analysis and quantification.

The indicators Nutrient cycling and Eutrophication index were used for the quantification of the NCP *Regulation of coastal water quality*, the Carbon footprint was used to estimate the NCP *Regulation of climate*, the Ocean acidification index indicator was applied to estimate the NCP *Regulation of ocean acidification*, and food and feed production were used for the NCP *Food and feed provision*. The performance of the selected LTS aquaculture systems was subsequently

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analysed and quantified using the selected indicators and NCPs, and the results were compared to each other, and to other common food production systems. Of the LTS evaluated in this report, sugar kelp production showed lower eutrophication mitigation potential than mussels and oysters and had a much lower farm-gate price compared to oysters and abalone, yet was found to be the only LTS to counteract ocean acidification. Bivalve production (mussel and oyster culture), on the other hand, was found to have a significant bioremediation capacity, with mussels displaying a higher capacity compared to all organism groups in this report. Compared to mussels, oysters obtained a higher farm-gate price, but also demonstrated a higher ocean acidification index, and the highest carbon footprint per unit of food produced among all LTS culture systems studied. In comparison, most mussel production systems had a lower ocean acidification index and a lower total carbon footprint per unit of food produced, than abalone and oysters. Abalone was the only LTS production system that showed a net release of nutrients, its ocean acidification index was set at the mid-range of values reported for oysters and at the upper range for mussels, and its carbon footprint per unit of food was similar to the higher values reported for mussels, while its farm-gate-price was considerably higher and only comparable to premium price oysters from Northern Europe. In terms of the total carbon footprint, mussels and abalone were found to have the lowest CF among the analysed shellfish production systems, equivalent to primary producers, oysters were found to be comparable to poultry and pig meat, but lower than e.g., lamb, beef, or other farmed marine species (e.g., fish and prawns), while sugar kelp had a residual carbon footprint and consequently may support climate change mitigation. It should be noted that all mussels, abalone, and even oysters may also indirectly support climate change mitigation e.g., by replacing other meat products. However, analysis of indirect effects of LTS aquaculture on the combined CF of food production around the Atlantic is not included in the scope of this study.

The quantification identified significant contributions to society, e.g., in terms of NCP *Regulation of coastal water quality*, as well as sustainable *Food and feed provision*. Some disservices were also identified, e.g., linked to the NCP *Regulation of climate* as the carbon footprint of shellfish culture was found to be in line with other traditional food sources, but generally lower than other meat products. In view of these results, a well-planned expansion of macroalgae and shellfish aquaculture together with campaigns that promote the consumption of these products over other meat products may contribute to cover the increasing demand for food in the world while mitigating eutrophication effects and reducing the current contribution of food production systems to the global GHG emissions. This includes developing context-dependent recommendations for expansion of LTS aquaculture where regional differences related to species and system performance are accounted for.

The results here presented will be integrated in the upcoming sustainability analysis of LTS aquaculture and will form the basis for the economic valuation of marine ecosystem services linked to LTS aquaculture in upcoming tasks from AV. Finally, identified knowledge gaps related to the link between some NCPs and LTS aquaculture, and data deficiencies are highlighted in this study to support future research needs in this field.

Acknowledgments

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Links

AquaVitae website: https://aquavitaeproject.eu

EUROPEAN CONSUMERS’ PERCEPTION OF FISH FARMING AND NEED FOR INFORMATION

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Introduction

For many consumers, aquaculture is not a well-known sector of food production (Fernández-Polanco & Luna 2012). To study the potential ways to increase the awareness, in Finland, France and Spain we assessed consumers’ perceptions on fish farming and on the information they receive and would like to receive. Our previous results showed that consumers receive a limited information about fish farming but are open to receive more (Latvala et al., 2021). The present work is focusing the perceptions of consumers about their knowledge on fish farming techniques and related fields, and their interest to learn about them.

Material and methods

To identify the key questions to be studied, during an initial workshop we collected the opinions of the AquaImpact program partners, researchers and professionals. Then a qualitative study was elaborated and carried out in five consumers focus groups in the three countries in 2019 (Mariojouls et al., 2021). Using the results of the qualitative study, we elaborated a quantitative study, carried out by online survey on a representative sample (415 people per country) in 2021. The master questionnaire included different parts, and we present here mainly results about farming practices. We asked questions such as “How do you think that farmed salmons in Europe are fed ? with…”, ”How do you think that farmed salmons in Europe are cared ? with...?”; How do you do you think that farmed salmons in Europe are reproduced and selected ? …”, proposing several techniques for every question, the respondent giving for each technique a level of agreement (always, occasionally, never, I don’t know). Some questions targeting on emerging techniques (in feed, genetics, RAS, fish welfare) concerned their acceptability.

Results and discussion

The limits of knowledge are reflected in the difficulty for answering certain technical questions, translated into consistent or high rates of «I don’t know».

The comparative approach between countries shows that there are significantly different response profiles in the three countries for most questions.

Regarding feeding practices, there are highly significant differences (at 0.1%) between countries for the two following techniques: the technique «with fishmeal» is considered effective (ratio of sum of shares for «always» and «occasionally» on total, after exclusion of “I don’t now”) by 95% of respondents in Finland, 92% in France, 74% in Spain where 65% choose “I don’t know” VS 23% in Finland and 34% in France, and “with a mixture of ingredients” considered effective by 93% in Finland, 94% in France, 89% in Spain, with shares of “I don’t know” at respectively 33%, 39% and 40%. For the other proposed techniques, the differences between countries were significant but less marked (at 5%). They were considered effective by 65 to 75% for “with little wild fish”, by 79 to 84% for “with plant ingredients” and by 71 to 81% “with animal meals”, and the shares for “I don’t know” are in the range of 37 to 47%. Finland shows the lowest share of “I don’t know” except in one case, meaning the best level of knowledge in salmon feeding.

Regarding care practices, we found highly significant differences between countries for all proposals (at 0.1%) except for ”with vaccines” (significant at 1%). The shares of answer “I don’t know” are high (41 to 54%) in all countries for three techniques (“vaccines”, “other medicines”, “cleaner fish to limit external parasites”), the lowest for “with high sanitary practices” (20 to 39%), and consistent for the others (29 to 54%), and for all techniques Finland has the lowest share meaning the best level of knowledge in care of farmed salmon. The method «with high sanitary practices» is considered effective at the highest level (Finland 96%, France 89%, Spain 94%) meaning a positive view of caring salmon in farms, while «with vaccines» is considered effective at the lowest level (Finland 66%, France 70%, Spain 64%) meaning a limited knowledge about the real use of this technique. Other techniques are considered effective by 71 to 90% of respondents.

The questions on reproduction and selection show that the shares for “I don’t know” are in a range of 35 to 58%, the highest being for the proposed technique “Mating of close relatives is avoided to prevent inbreeding”. We found again the lowest shares of “I don’t know” in Finland, meaning a better knowledge in fish breeding than in France and Spain. The technique “males and females mate naturally” is considered effective by the lowest shares (Finland 76%, France 73%, Spain 85%), while other techniques are at high levels (82 to 92%) except “Mating of close relatives is avoided to prevent inbreeding” with great discrepancies between Finland (82%) VS France (45%) and Spain (40%), confirming a better knowledge in Finland.

Questions about acceptance of emerging techniques in feeding had rather limited shares of “I don’t know” (23 to 31%). In total panel, the acceptance (sum of « totally acceptable » and « weakly acceptable » on total incl. “I don’t know”, in whole panel) is high (52 to 63% according to techniques) for feed containing insects, micro-algae, only plant ingredients, only local ingredients, but more limited for feed containing oil rich in omega 3 from GM seeds (45%), and feed containing authorized animal meals (42%), and low only for feed containing yeasts (34%). About genetics, « I don’t know » is expressed by a limited share of respondents : 22% to 26 %. There is a good or very good acceptance for traditional selection (67%) and genomic selection (58%), and unsurprisingly, GMO shows the lowest acceptance (29%), as found in previous research (Barrey et al., 2011). For other emerging techniques, « I don’t know » is expressed by a limited share of respondents (21 to 25 %), and the acceptance is high for proposal on improvement of fish welfare (67%) and for RAS technology (62%). Between countries there are significant differences for all proposed techniques at 0.1%, except for feed containing local ingredients (at 5%), and no significant differences for proposals on GMO, fish welfare, RAS.

Consumers in all countries consider that the information received today is insufficient, and that receiving information would be really useful at high rates, from 73 to 91% (sum of shares for “slightly useful”, “useful and “very useful” on total by country) with lesser levels in France, about a wide range of topics (farming practices, regulation & controls, nutritional content, economics) with various levels of interest for these topics according to countries.

Conclusion : Our results suggest the need for dissemination to consumers of information on fish farming, in forms adapted to the subject and the population of each country.

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CULTIVABLE BACTERIAISOLATED FROM TWO BIVALVE SPECIES OF ECONOMIC AND FOOD INTEREST - THE SEEBug PROJECT

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Introduction

Several contaminants affect the productivity and safety of bivalve fisheries sectors, such as phytoplankton-produced biotoxins, metals and microbiological agents (Shi et al. 2016; Blanco, 2018; Martin et al. 2019). Pathogenic microorganisms are commonly associated with foodborne diseases in humans from shellfish consumption and can be caused by a variety of bacterial genera (Grevskott et al. 2017). In order to protect consumers from these pathogens, methods like depuration are used to reduce the content of microbiological contaminants in bivalves (Polo et al. 2015). Nonetheless, there are bacterial strains capable of resisting the depuration process, potentially causing problems to public health (Martínez et al. 2009). The aim of this work, which was conducted under the research project SEEBug (Development of a sensor for the fast and efficient detection of pathogenic bacteria in Bivalves; funded by MAR 2020) is being to isolate and identify the cultivable bacteria associated with two bivalves species.

Materials and methods

Specimens of depurated and non-depurated *Venerupis Corrugata* and non-depurated *Mytillus* sp. were collected in Ria de Aveiro (Aveiro, Portugal), in July 2021. The microbiological analysis was performed according to two different methodologies: P1 - The processing, extraction, and detection of *Escherichia coli* in bivalves species following standardized procedures (ISO 6887-3:2017, ISO 16649-3:2015 and ISO 7218:2007) in compliance with the approved Regulations, namely Reg. (CE) N.º 854/2004, Reg. (CE) N.º 1021/2008 e Reg. (EU) 2015/2285). MPN values were calculated to assess the health status of processed bivalves and production zones; and P2 - MacConkey Agar (MC) and Thiosulfate-citrate-bile salts-sucrose (TCBS) served as selective culture media and Tryptic soy agar (TSA) as a non-selective medium to obtain bacterial isolates, to enable the counting of colony forming units (CFUs) and the preliminary description of the cultivable microbial diversity present in the shellfish biomass. Molecular techniques (amplification and sequencing of 16S rRNA gene) were applied for the taxonomic identification of the isolated bacteria.

Results

By following the standard protocols in P1 it was possible to successfully identify *E. coli* from non-depurated Pullet carpet shell (*V. corrugata*) individuals. The MPN values obtained are within the legal limit set for the health status class C (> 4.600 to ≤ 46.000 E. coli MPN / 100 g), according to Reg. (CE) No. 854/2004, Reg. (CE) No. 1021/2008 and Reg. (EU) 2015/2285. Regarding the P2 methodology, several genera of cultivable bacteria were detected in both bivalves. Cultures on MacConkey agar showed a greater number of bacterial isolates and therefore higher CFU values. Molecular methodologies allowed the identification of eight bacterial genera (*Aeromonas* sp., *Escherichia* sp., *Photobacterium* sp., *Pseudomonas* sp., *Serratia* sp., *Shewanella* sp., *Klebsiella* sp. and *Providencia* sp.).

Conclusion

Through the detection of *E. coli* isolates, it was confirmed that the application of ISO protocols are effective in determining the level of *E. coli* in for regulatory purposes. The MPN values demonstrated that the depuration process reduces the concentration of viable *E. coli* in bivalves. However, the health status remained the same for *V. corrugata*. In this case, the depuration period would probably have to be longer to decrease the MPN values, and thus contribute to the consumers' food safety. The cultivation of bacteria isolated from bivalves, on selective and non-selective media, constituted a first approach to the diversity study of the cultivable microbial community and to identify *taxa* that are commonly considered pathogenic (belonging to Pseudomonadaceae, Aeromonadaceae, Vibrionaceae and Enterobacteriaceae). Obtaining these pathogenic isolates will enable the development of early detection sensors in bivalves of economic interest.

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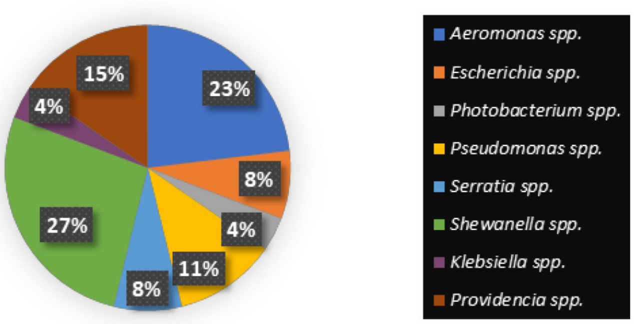

Figure 1 - Bacterial genera identified in *V. corrugata* and *Mytilus* sp. with their overall percentages (%).

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DIETARY AND SALINITY MODULATION OF THE FATTY ACID PROFILE OF THE EURYHALINE TELEOST THICKLIP GREY MULLET (*Chelon labrosus*)

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Introduction

The need to replace fish oils (FO), rich in long-chain (C20-24) polyunsaturated fatty acids (LC-PUFA), by vegetable oils (VO), devoid of LC-PUFA but rich in their C18 precursors, has led to a growing interest in understanding how the LC-PUFA biosynthetic pathways can be regulated in fish species of commercial importance. *Chelon labrosus* is an euryhaline teleost able to live in environments of different salinities such as estuaries and coastal waters. *C. labrosus* has been proposed as a good candidate for aquaculture diversification due to its omnivorous profile, rapid growth and resistance to environmental variations (Abellan and Arnal, 2013). Moreover, the functions of the LC-PUFA biosynthesizing enzymes fatty acyl desaturase (Fads2) and elongase (Elovl5) from *C. labrosus* has recently reported, demonstrating its capacity to produce the physiologically essential 20:5n-3 (EPA) and 22:6n-3 (DHA) from the C18 precursor 18:3n-3 (ALA) (Galindo et al., 2021). These characteristics make this species a perfect model to evaluate dietary and salinity modulation of EPA and DHA endogenous production. The present study assessed the combined effects of two dietary LC-PUFA contents and two salinities in the regulation of LC-PUFA biosynthesis in *C. labrosus* juveniles through the modification of the muscle fatty acid profiles and the regulation of the expression of *fads2* and *elovl5* genes in liver.

Material and methods

C. labrosus juveniles with an average body weight of 14.6 ± 1.6 g were randomly distributed into four quadrangular flat bottom 1000 L-tanks (15 fish each) and reared for 10 weeks in an aquaponic system at Fundación Neotrópico (Tenerife, Spain) facilities, at a temperature of 19.5 ± 0.4 °C, and under a natural photoperiod regime. Fish from two tanks were fed a FO-based diet (TI-5 Tilapia; Skretting + 4% Croda's Incromega™ oil), while the other two tanks received a VO-based diet (TI-5 Tilapia; Skretting + 4% olive oil). For manufacturing both experimental diets, the commercial TI-5 Tilapia feed was crumbled, the oil added, and the mixture finally repelletized. FO diet contained 6-fold more DHA and near 2-fold higher EPA than the VO diet. Fish were fed twice daily at a rate of 3-5% of their total biomass. Each diet was tested at 35 ppt and 20 ppt, resulting in four experimental treatments: FO35, FO20, VO35 and VO20. At the end of the experimental period, fish were individually measured and weighed, and subsequently pooled into five groups of three fish (n=5) per tank. Pooled samples of muscle were collected for fatty acid analysis. In addition, a portion of liver (~100 mg wet weight) was collected in RNAlater®, stored for the first 24 h at 4 ºC and then frozen at -20 ºC until further analysis of gene expression of the *C. labrosus fads2* and *elovl5* genes by qPCR. *ef1α* and *β-actin* were used as reference genes to normalize the expression of *fads2* and *elovl5*.

Results

Survival was 100% in all experimental groups. No significant differences in fish growth and total lipid content of muscle were registered among treatments. In addition, muscle EPA contents did not vary between fish groups. In contrast, FO20-fish displayed the highest amount of DHA. Relative expression in liver showed greater effects of salinity than dietary regime, with both *fads2* and *elovl5* being upregulated in fish reared at 20 ppt.

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Discussion and conclusions

The present study showed that *fads2* and *elovl5* in liver were upregulated in fish reared at 20 ppt, indicating that salinity is a key parameter in the expression of these genes, independently of the diet. These results are consistent with other studies in marine teleosts such as *Pagrus major* or *Siganus canaliculatus* reared at a lower salinity than that of seawater (Sarker et al., 2011; Xie et al., 2015). Indeed, low salinity has been reported to increase the content of LC-PUFA in other mugilid species such as *Mugil cephalus* (Khériji et al., 2003). LC-PUFA biosynthesis can also be stimulated with low dietary levels of LC-PUFA in combination with changes in the environmental salinity (Marrero et al., 2021). In fact, a higher hepatic expression of both enzymes at low-salinity was found, albeit this only implied a difference in the amount of DHA levels in FO-fish flesh. Meanwhile, VO-fish showed no significant differences between salinities with respect to this omega-3 LC-PUFA, despite VO20-fish being upregulated for both genes. In conclusion, *C. labrosus* was able to compensate the lower dietary input of DHA with respect to the control treatment (FO35) despite VO diet provided 6-fold less DHA and 2-fold less EPA than FO diet.

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DNA VACCINES ARE EFFICACIOUS IN PROTECTING RAINBOW TROUT (*Oncorhynchus mykiss*) FROM VHS AND IHN: EXPERIMENTAL AND FIELD EVIDENCES

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Introduction

Viral Hemorrhagic Septicemia (VHS) and Infectious Hematopoietic Necrosis (IHN) have a great impact on the Italian rainbow trout farming industry and the availability of vaccines is a key point in controlling and reducing their effect on productions. DNA vaccines have proved to be effective in protecting rainbow trout from VHS and IHN in controlled settings, but no data are available about their efficacy in intensive rainbow trout farming conditions.

Materials and methods

Italian VHSV and IHNV glycoprotein gene sequences were used to design two DNA vaccines tailored towards current virus variants causing disease in rainbow trout farms in Italy. An experimental trial was first conducted at the IZSVe Experimental Aquarium in order to obtain efficacy and safety baseline data. Water temperature was set at 12°C along the whole trial. Ten-gram rainbow trout (*Oncorhynchus mykiss*) juveniles were treated with 0.1 or 1 mg/fish of the plasmids, injected singularly and combined. Fish were bath challenged 60 days post vaccination (dpv) with two recently isolated Italian VHSV and IHNV, singularly and in combination at the dose of 10^4 TCID50/ml. Cumulative mortality in control groups resulted to be 85.2%, 38.2% and 92.4% for IHN, VHS and coinfection respectively. Dose of 1 mg/fish produced the highest RPS and was selected for the field trial. Due to lower mortality in the IHN control group and the farm epidemiological situation, only the VHS and the VHS/IHN treatments were selected for the field trial.

One Category I hatchery supplied 15,000 rainbow trouts weighing approximately 8 grams that were then divided into three experimental groups of 5,000 fish each: one injected with PBS (Group 1), one injected with 1 mg/fish of VHS vaccine (Group 2) and the last group treated with 1 mg/fish of each plasmid mixed together (Group 3). Fish were then moved to a VHS/IHN infected facility 60 days post vaccination and positioned in a single raceways divided by grid. For 60 day post transfer (dpt), symptomatic fish from Group 2 and 3 were individually collected and subjected to qPCR for VHSv and IHNv in order to identify the viral species causing mortality in these groups. In Group 1, fish were monitored in pool daily or weekly once mortality began due to the high number of fish which died for 60 dpt. Afterward, anatomopathological examinations were performed in all the three groups monthly or in case of variation of the mortality trends. At 270 dpv fish reached the marketable size and were destined to human consumption and the field trial ended.

Results

Mortality began 7 days post transfer (dpt) with different trends in the three groups. In Group 1, VHS resulted to be responsible for the mortality in the first 30 dpt and was replaced by IHN which persisted along all the trial, causing low and constant mortality. No bacterial pathogens were identified except for *Yersinia ruckeri* biotype II which caused mortality in all the three experimental groups between 200 and 240 dpv. Based on antibiogram, medical treatment was performed *per os* for 10 days and proved effective. At 30 days post transfer, mortality resulted to be 10.5 % in Group 1, 3.3% in Group 2 and 1.0% in Group 3, with a RPS of 69.3% and 90.5% provided by VHS and VHS/IHN vaccine respectively. At 60 dpt, RPS decreased for the VHS vaccine only (59.6%) due to the mortality caused by IHN, while the VHS/IHN vaccine maintained the same level of protection (87.1%).

At the end of the field trial (270 dpv), cumulative mortality was 35.3% in the control group, 26.0% in the VHS-vaccinated group, and 20.0% in the bivalently-vaccinated group and the mean weight was approximately 430.7±143.7 grams, 404.1±106.4 grams, 429.9±110.5 grams in Group 1, 2 and 3 respectively confirming that tested vaccines didn't produce any adverse zootechnical effect.

Conclusions

DNA vaccines confirmed to be safe for injected fish and effectively reduced the impact of VHS and IHN also under field conditions.

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EFFECTS OF MICRODIETS ENRICHED WITH THE MICROALGAE *Nannochloropsis gaditana* ON MUSCLE COMPOSITION, OXIDATIVE CONDITION AND DIGESTIVE FUNCTIONALITY OF SEA BREAM (*Sparus aurata*) LARVAE

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Introduction

Early life stages of marine fish are critical to the morphological and physiological changes that determine their further viability thorough their productive cycle. In this regard, the inclusion of functional additives in microdiets is currently a hot topic in aquaculture research. Among the wide variety of those functional additives, microalgae have proven their value owing to their richness in biostimulant compounds. Nevertheless, some microalgae species rich in these bioactive substances have a thick cell wall that might impair their release, and therefore, their physiological effects (Wu *et al*., 2017). Even if raw microalgae biomass is routinely added to weaning diets, however, when the potential biological effects depend on microalgae inner compounds, a pre-treatment might increase their bioavailability. In this regard, enzymatic hydrolysis may represent a useful strategy aimed at weakening the microalgae cell wall, thus increasing the release of bioactive compounds. In this context, we hypothesize that low dietary inclusion level (up to 5%) of *N. gaditana* either crude or hydrolysed, might improve the digestive functionality of larvae. With this purpose, a 45-d feeding trial was set out, after which several parameters related to body composition, antioxidant status, and digestive morphology and physiology of gilthead seabream (*Sparus aurata*) larvae were assessed.

Materials and methods

Nannochloropsis gaditana was cultured in tubular photobioreactors at the pilot plant (EU-H2020 SABANA facilities) of the Universidad de Almería (Spain). Subsequently, enzymatic hydrolysis of raw algal biomass was carried out as reported in Ayala *et al.* (2020). Five isonitrogenous and isolipidic (46% and 16% DM, respectively) experimental diets were elaborated at the CEIA3-University of Almería facilities (Service of Experimental Diets). Two inclusion levels (25 and 50 g kg^{-1} w/w), and two microalgae formats (raw and enzymatically hydrolysed) were considered in 0.2 to 0.4 mm microdiets. Therefore, diets were designed as R25 and R50 for raw microalgae lots, and H25 and H50 for diets containing enzymatically-hydrolysed biomass. A microalgae-free diet was used as control (CT). Gilthead sea bream larvae were randomly distributed in 15 tanks and fed daily at 5% of their biomass. At day 22 and 45 of the feeding trial, fish were sampled for determinations of muscle proximate composition and lipid oxidation status by thiobarbituric acid reactive substances (TBARS). In addition, some digestive enzymatic activities, transcriptomic profiling of intestinal markers and the study of the intestinal mucosa by scanning electron microscopy (SEM) were evaluated.

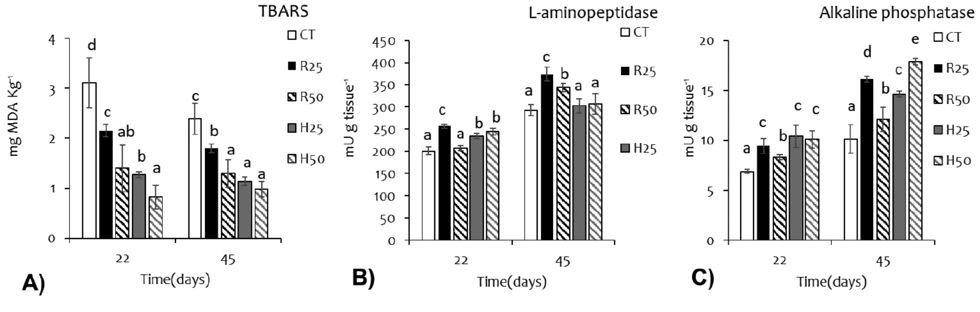

Figure 1. A) TBARS content in *S. aurata*. B and C) Digestive enzyme activities measured on intestinal extracts after 22 and 45 d of the feeding trial. CT: controls. R25 and R50 stand for 25 and 50 g Kg^{-1} of raw algal biomass, respectively. H25 and H50 stand for 25 and 50 g Kg^{-1} of hydrolyzed algal biomass, respectively. Values are mean ± sd (n=15). Different lowercase letters indicate significant differences ($P<0.05$).

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Results and discussion

Overall, the use of *N. gaditana* as functional additive in diets did not cause any difference in proximate analysis. Nevertheless, *N. gaditana* meal at low inclusion level in microdiets improved the antioxidant response ($p<0.001$) compared to CT fish. This effect was more evident when microalgae biomass was pre-treated with cellulase (H25 and H50, $p< 0.001$) at any sampling time (Figure 1). It is likely that the richness of *N. gaditana* in natural antioxidants (carotenes, polyphenols, etc.) and other bioactive compounds underpins these effects on lipid oxidation.

On the other hand, at 22 d animals fed on CT, R25 and R50 diets showed significantly higher trypsin, chymotrypsin and total alkaline protease enzyme activities. However, this effect was less evident after 45d, time at which the specific enzyme activities measured did not differ among dietary treatments. With regard to brush border enzymes (alkaline phosphatase and leucine aminopeptidase), all the microalgae-enriched diets increased their activities, compared to CT. However, such increase dependent more clearly on the inclusion level than on biomass pre-treatment. These enzyme activities play a fundamental role in the final stages of digestion, allowing the absorption and transport of nutrients through the enterocytes, and they are used as indicators of the intestinal integrity or as general markers of nutrient absorption. The intestinal mucosa plays a key role not only in digestion and absorption processes, but also as a protective barrier against pathogenic microorganisms. These results are in agreement with gene expression analyses of intestinal markers, and also with the findings after the electron microscopy studies of the intestinal epithelium.

Conclusions

The results obtained indicate that the inclusion of *N. gaditana* meal at low level (25 and 50 g kg^{-1}) in microdiets had an impact on different aspects related to antioxidant activity and certain digestive functionality parameters of sea bream at initial stages of their productive cycle. The enzymatic pre-treatment of the microalgae with celullase enzymes can increase the antioxidant effects, but no influence was observed in most of the parameters related to the intestinal functionality. Further research assessing the potential effects on intermediary metabolism and fish immune response are needed as well.

Acknowledgements

This research was funded by MINECO-FEDER (grant # RTI2018-096625-B-C33 and grant # RTI2018-096625-B-C31, AquaTech4Feed (grant # PCI2020-112204) granted by AEI within the ERA-NET BioBlue COFUND, and SABANA project (EU-H2020, grant # 727874).

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INFLUENCE OF *Nannochloropsis gaditana* ENZYME HYDROLYSATES INCLUDED IN FINISHING DIETS ON THE SHELF-LIFE OF GILTHEAD SEABREAM (*Sparus aurata*) FILLETS

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Introduction

Nannochloropsis gaditana is a microalgae species characterized by having adequate protein content, as well as valuable amino acid profile, together with significant concentration of liposoluble vitamins and carotenoids with antioxidant properties, so that its inclusion as feed additive at low concentration might well be promising in the context of fish fillet preservation (Sales *et al.,* 2021). Nevertheless, the existence of a cellulose-rich cell wall could limit the *in vivo* bioavailability of the bioactive intracellular components when *N. gaditana* is included in aquafeeds. For the abovementioned reasons, simple, economical, or at least, cost-effective cell-wall disruption protocols, aimed at easing industrial scaling, are still needed. In this regard, the use of hydrolytic enzymes capable of weakening *N. gaditana* cell walls, namely cellulases, prior to its incorporation into feeds is among the most feasible strategies. Bearing in mind previous premises, the bioavailability of the intracellular compounds of *N. gaditana* might be improved, compared to crude microalgae, by pre-treating enzymatically the biomass, compared with crude microalgal biomass. Moreover, this increased release could improve objective quality parameters and shelf-life of cultured gilthead seabream (*Sparus aurata*) fillets when hydrolysates are included finishing diets for this fish species.

Materials and methods

Nannochloropsis gaditana was obtained from EU-H2020 SABANA facilities of the Universidad de Almería (Spain). Five iso-nitrogenous (45.5%, DW) and iso-lipidic (15.5%, DW) experimental feeds were formulated; two of them contained 25 and 50 g kg-1 *N. gaditana* raw biomass (labelled as R25 and R50, respectively); other two experimental groups included 25 and 50 g kg^{-1} *N. gaditana* hydrolysates (designated as H25 and H50, respectively), and a fifth diet, microalgae-free, was used as the control batch (CT). The feeding trial was carried out at the aquaculture facilities of Centro Oceanográfico de Murcia (Mazarrón, Spain), Instituto Español de Oceanografía (IEO-CSIC). Gilthead seabream (400 g average initial body weight) were randomly distributed in 15 tanks (triplicate tanks per dietary treatment). Fish were fed with CT diet (microalgae-free) during a 15-d acclimation period prior to the beginning of the feeding trial. Afterwards, the experimental diets were offered at 2% of the biomass during a 42-d period. At the end of feeding assay, 10 fish per tank (30 animals per dietary treatment) were withdrawn. Immediately after slaughtering, specimens were gutted, filleted and packed in transparent sterile polyethylene bags. These bags were directly stored at 4 ºC in a cold room (4 ºC ± 1 ºC) for a period of 15 d with the aim of assessing changes in quality parameters throughout the fillet shelf life. Samples were extracted from each lot at 1, 2, 4, 7, 9, 11 and 15 days post-mortem (dpm), and total viable counts, lipid oxidation, texture profile analysis (TPA), pH, water holding capacity (WHC), skin pigmentation were determined at each sampling time.

Results and discussion

At the end of the production cycle, feeding can play a key role in the final quality of the final product. The results obtained indicate that microalgae supplement ($P<0.01$) and storage time ($P<0.01$) were responsible for significant differences in psychrophilic bacterial counts (PBC). However, from 7 days *post-mortem* (dpm) onwards microalgae inclusion lowered microbial growth up to 15 dpm compared to CT batch. However, neither microalgae dose ($P=0.581$) nor enzyme pre-treatment of the biomass ($P=0.989$) affected bacterial counts.

Lipid peroxidation is one of the main concerns during fish storage, as it contributes to quality deterioration and shortens fillet shelf life. In this regard, microalgae supplementation in aquafeeds has shown positive effects, slowing down lipid oxidation phenomena (Shah *et al.,* 2018). In this assay, from 4 dpm onwards CT fillets yielded higher TBARS values compared to any of the batches fed with *N. gaditana*-containing diets ($P<0.01$). In addition, a dose-dependent effect was observed, with lower values in R50 and H50 fillets, although, these differences became significant only at later stages of the storage period (after day 11). Concerning fillet hardness, overall, the inclusion of *N. gaditana* in diets yielded higher values

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(P<0.01) for this parameter compared to CT fillets, especially for 5% inclusion level. In addition, Also, the dietary inclusion of raw biomass tended to yield fillets with higher hardness than those obtained from fish fed with the hydrolysed biomass, although differences didn't reach statistical significance (P=0.069). Roughly, springiness, cohesiveness, gumminess, and chewiness followed a tendency similar to that observed for hardness. Also, pH was influenced by both dietary treatment and dosage, and thus, higher *N. gaditana* inclusion level (R50 and H50) yielded lower values (P<0.01 in both cases). On the other hand, WHC, which represents the percentage of water retained in the muscle, was not affected by *N. gaditana* inclusion (P=0.055), even if average values tended to be lower in CT batch than in H50 and R50 fillets. Skin colour is a key parameter influencing consumer acceptability. At the beginning of the storage period, dorsal skin of microalgae-fed lots showed higher values for L* (lightness) and b* (yellowness), but lower for a* (greenness) than CT fillets. Nevertheless, during cold storage time, L* decreased significantly in microalgae-fed lots throughout the 15-d period, with similar final values in all batches. On the other hand, a* parameter decreased in all fillets, although microalgae-fed groups yielded consistently lower values (more greenish) than CT. Finally, b* parameter also decreased owing to storage time in all experimental groups, being values for this parameter consistently higher in R50 and H50 fillets compared to CT lot up to 11 dpm.

Conclusions

The results obtained indicated that the inclusion of *N. gaditana* meal in diets at low concentration (25 and 50 g kg^{-1}) in finishing diets influenced different organoleptic parameters, improving textural properties and maximizing skin pigmentation. Fillet shelf-life was also affected by microalgae supplementation, yielding lower values of lipid oxidation and psychrophilic bacterial counts. Nevertheless, enzymatic pre-treatment of *N. gaditana* biomass did not have a significant impact on these parameters.

Acknowledgements

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VINIFICATION BY-PRODUCTS AS A SOURCE OF BIOACTIVES PRODUCTS OF INTEREST IN AQUACULTURE

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Introduction

Currently, aquaculture is based on the use of intensive systems that are characterized by increasing environmental stress agents and reduced water quality what facilitates the appearance of pathologies in individuals. Taking this into account, more attention is being paid to the inclusion of ingredients with biological activity that can improve fish health status. In the context of the circular economy, it is of great interest to evaluate the potential of many agri-food by-products as important source of bioactive products with antioxidant capacity (terpenes, carotenoids, phenolic compounds, etc.). Among these, it is worth mentioning the wine by-products (WB) such as grape pomace and lees, which have traditionally been used in feeding terrestrial animals, but whose application in aquaculture is yet to be developed. However, some of these compounds like polyphenols, that present interesting properties as antioxidants, may also interact with other nutrients during digestion, modifying their bioaccessibility and hence their potential bioavailability. In the present study some of these aspects were evaluated through two experiments aimed to asses: a) the possible negative effect that phenolic compounds present in WB may exert on the digestive proteases of a marine fish (the seabream *Sparus aurata*) and b) how inclusion of such phenolics within the feed matrix may affect potential bioavailability of protein during the digestion process. This last experiment was developed by *in vitro* assay simulating the digestion in two fish species, the sea bream (*S. aurata*) and the grey mullet (*Mugil cephalus*).

Materials and methods

The WB used in the different experiments (red grape pomace and lees) were obtained from two artisanal wineries placed in Almería and Cádiz, (Spain) respectively. The grape pomace was dried in an oven for 72 h at 60 ºC and subsequently finely grounded until obtaining a fine powder that was used in the assays while the lees were employed in liquid form in all tests. A complete characterization of phenolic compounds and antioxidant capacity was carried out in both WB using specific methods (Brand-Williams et al., 1995; Graça et al., 2005). Two different experiments were developed:

Experiment 1. An inhibition assay was carried out by preincubating digestive enzyme extracts obtained from seabream, with a known protease activity, with variable amounts of grape pomace meal and after measuring the resulting reduction of activity compared to a control sample.

Experiment 2. This experiment was oriented to assess the potential negative effect of WB phenolics on protein bioavailability when the product was included at 10 % (w/w) in a matrix composed by hemoglobin, starch, sunflower oil and carboxy methyl cellulose designed to simulate a compound feed. The assay was developed using a membrane bioreactor described in Gilannejad et al. (2017) adapted to simulate *in vitro* the digestive conditions of two farmed fish species (seabream and mullet). Total amino acids released during the hydrolysis were measured using the o-phtaldialdehyde method (Church et al., 1983) and total phenolics were determined by the method described by Graça et al. (2005).

Results and discussion

The analysis of WB showed a significantly different profile of phenolic compounds (Table 1). The content in all the types of phenolic compounds was always higher in grape pomace than in lees, while the antioxidant capacity measured in the former product was higher. Results obtained in the inhibition assay are detailed in Figure 1. The inhibitory effect was higher on acid than in alkaline protease activity. The curves were used to estimate the expected inhibition on such activities resulting from the intake of a feed containing 10 % WB in a 100 g fish, these accounting by 7 % and 4 % for acid and alkaline proteases, respectively.

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Table 1. Values are presented as mean SD. Values in columns not sharing the same letter differ significantly with $p < 0.05$. Comparisons between by-products are indicated with lowercase letters.

By-products	Antioxidant capacity (μmol TEAC/g d.m.)	Total phenolics (mg galic acid/g d.m.)	Flavonoids (mg rutin/g d.m.)	Tannins (mg tannic acid/g d.m.)	Anthocyanins (mg cyanidin-3-glucoside/g d.m.)
Lees	134.44 ± 15.96^a	26.27 ± 1.10^a	15.44 ± 1.71^a	13.58 ± 0.70^a	0.251 ± 0.00^a
Grape pomace	337. 37 ± 14.05^b	65.88 ± 0.85^b	46.49 ± 2.38^b	21.71 ± 1.33^b	0.160 ± 0.01^b

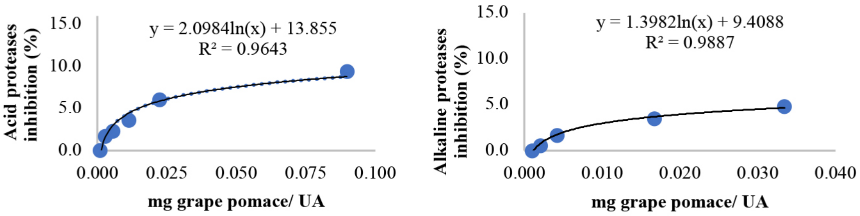

Figure 1. Inhibitory response of seabream digestive proteases (stomach and intestine) after incubation with increasing doses of grape pomace meal.

Table 2. Nutrients released after *in vitro* hydrolysis in Experiment 2. Comparisons between species are indicated with capital letters.

	By-products	mg total phenolics/g by-products	Percentage reduction in amino acid bioavailability
Seabream	Lees	2.87 ± 3.09 A	36.86 ± 2.68 A
	Grape pomace	2.65 ± 3.75	32.04 ± 7.55
Mullet	Lees	12.08 ± 3.04 B	21.94 ± 3.45 B
	Grape pomace	6.36 ± 2.56	16.16 ± 0.70

Data of the release of amino acids and phenolic compounds due to the action of the digestive enzymes of seabream and mullet under conditions simulating the digestion is summarized in Table 2. A significant reduction in the amount of amino acids release, ranging from 16 % to 36 %, was evidenced when any of the two WB were present in the feed mixture when compared to control diet (WB free). In addition, significant differences were obtained between species with a lower reduction in the case of mullet (no acid digestion). No significant differences were observed in results obtained with the two by-products (pomace and lees) within each species. Results suggest that the use of WB as a source of bioactive phenolics can produce negative effects on protein digestion of fish.

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AMINO ACIDS COMPOSITION AND PROTEIN QUALITY EVALUATION OF RAW MATERIALS FOR FEED FORMULATIONS IN MULLETS

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Amino acid composition was analyzed in three species of mullets, and, in different commercial raw materials (from animal or plant origin) and new alternative protein sources, in order to evaluate their possible use as ingredients for mullet diets. A total of 45 raw materials were analyzed, being afterwards clustered according to their protein suitability. Commercial plant and marine proteins presented the lowest and highest protein quality, respectively, meanwhile animal proteins showed a high level of a high AAR, but very low of CS. Finally, considering the raw material evaluated the most limited AA for mullet diet formulation was Met, followed by His.

Introduction

Mullets are excellent candidates to promote the aquaculture diversification, due to their omnivorous diet, excellent nutritional and organoleptic quality, and the possibility to produce them in diverse production systems and environments. Among the species of mugilids, the *Liza aurata*, *Chelon labrosus* and the *Mugil cephalus* are some of the species with commercial interest. However, due to its recent attention in its production, the information available on the nutritional needs, as well as, formulation of specific diets for these species is scarced or practically zero. Therefore, the objective of present work was to determine the suitability of different protein sources as potential components in the formulation of feed for these three species of mullets.

Material and Methods

For the present work, 45 protein sources (PS) were evaluated, which differed into: marine, new alternative animal and plant sources and also 10 specimens of each three mullets species (*Liza aurata*, *Chelon labrosus* and *Mugil cephalus)* with an average weight between 2 and 5 grams from extractive fishing. Proximate composition of raw materials and whole body fish were analysed in triplicate according to AOAC (1997). Following the method previously described by Bosch et al. (2006), amino acids (AA) of the samples were determined using a Waters HPLC system. With the AA data obtained, the Amino acid ratio (AAR, %) was calculated as (AAsample)/(AAreference) x 100, where AAsample and AAreference are the amino acid contents in the test sample and whole mullet (in each species), which was taken as reference, being the Chemical Score (CS, %) the minimum value from AARs calculated for essentials amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val). In addition, Oser index was calculated follows the equation (Oser, 1951):

Results and discussion

Figure 1 shows the cluster of raw materials according to their AA adequacy for each mullets species analysed, considering three parameters: AAR, CS and IO. The PS were classified into 4 well-differentiated groups. Plant proteins registered low values for the three parameters (green), therefore, less suitable as protein source. On the contrary, marine proteins reported the most suitable profile with high values in all parameters (in orange). A third group correspond to mainly animal protein showed a high AAR, but very low of CS (in blue), and, finally, a mix of plant and animal protein sources registered a very low of IO (in yellow). On the other hand, when the AA profile of raw materials is compared with the mullets whole body, the most limiting AA for protein synthesis was Met, followed by His. In general, the AAR of animal proteins were rich in Arg and Thr, low in Iso, Leu, and particularly low in Met and Lys AAR, as has been observed in the alternative animal and plant proteins. Nevertheless, alternative protein source exhibited a high percentage in Arg (except fermented rice). This study is essential to select the most appropriate combination of ingredients for mullet diets formulation.

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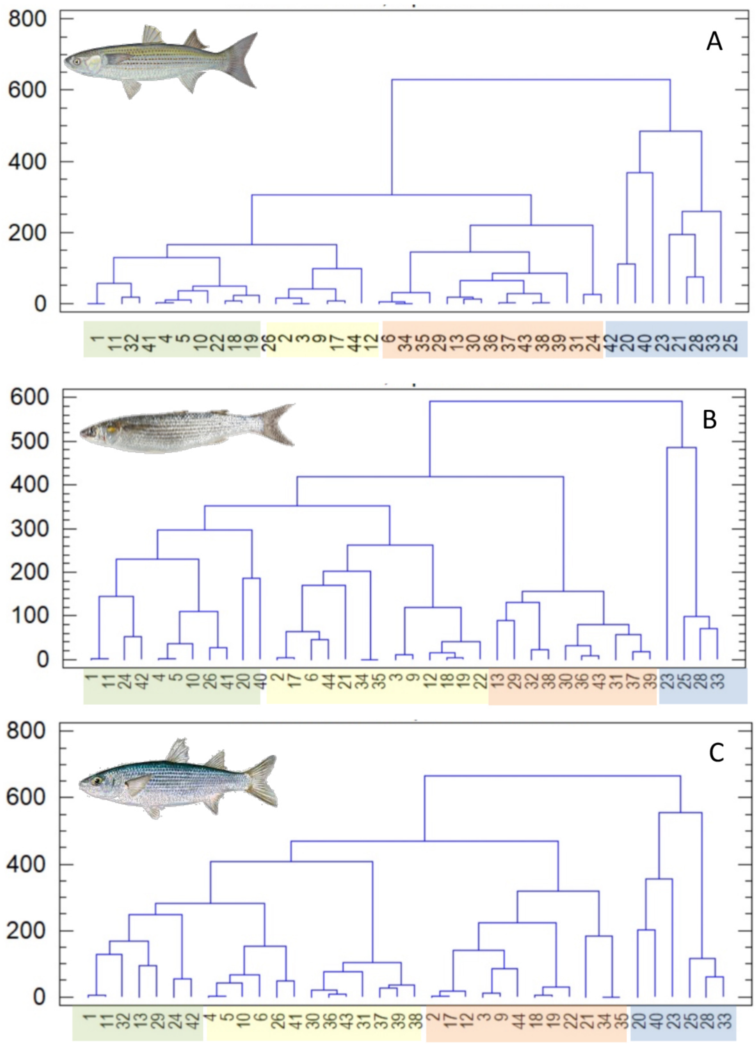

Figure 1. Dendrogram resulting from the cluster analysis where the different raw materials are grouped according to the RAA, the CS and the Oser index, for each of the species analyzed (A. *Mugil cephalus*; B *Liza aurata* y C. *Chelon labrosus*). *Wheat, 1; Beans, 2; Camelina, 3; Wheat bran, 4; Wheat bran, 5; Rice bran, 6; Beet pulp, 7; Fermented soybean, 8; Soybean, 9; Corn, 10; Sunflower, 11; Pea concentrate 75%, 12; Pea concentrate 55%, 13; Soybean concentrate, 14; Hydrolyzed potato protein, 15; Hydrolyzed wheat protein, 16; Pea, 17; Pea Protein, 18; Defatted soybean, 19; Corn gluten, 20; Carob seed, 21; Conc. Soybeans, 22; Wheat Gluten, 23; Rice Protein, 24; Iberian Pork, 25;Poultry meal, 26; Meat, 28; Fishmeal, 29; Fishmeal LT, 30; Krill, 31; Squid meal, 32; Squid, 33; Whole crab,34; Crab without shell, 35; Isochrysis galbana 10%, 36; Nannochloropsis Gaditana Premium, 37; Schizochytrium mangrovei, 38; Lemma, 39; Vinegar, 40; Citrus Pulp, 41; Fermented Rice, 42; Daphnia, 43; Worm, 44; Brewer's Yeast 100, 45;*

Acknowledgments
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THE IMPORTANCE OF A COMMON METHODOLOGICAL APPROACH FOR THE ASSESSMENT OF THE CARBON SINK POTENTIAL OF MOLLUSC FARMING

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Introduction

The need to make food production more sustainable and allow access to high nutritional value food for the entire population is nowadays a firm awareness. Thus, it is of paramount importance to quantify the positive and negative environmental impacts of food supply chains to foster the ecological transition towards those having zero or positive impacts and that might also have a significant role in the mitigation of climate change. Bivalve mollusc aquaculture provides high-quality animal proteins and omega-3 PUFA, and is characterized by one of the lowest carbon footprints in the animal production panorama. Furthermore, it can mitigate the effects of eutrophication and play a role in the storage and cycle of fundamental nutrients in aquatic environments. These ecosystem services of bivalve farming are well established. Differently, carbon sink potential is at the centre of an intense scientific debate. Shell formation occurs through the chemical reaction between calcium ions and dissolved carbon dioxide. The reaction produces calcium carbonate, which is incorporated into the shell, and carbon dioxide, released into seawater. Therefore, why is the biocalcification process often considered a carbon sequestration mechanism? The aim of this work is to stimulate a discussion around this complex and debated topic. Data on the biogenic carbon flows and the carbon sequestration potential of biocalcification in farmed Mediterranean mussel (*Mytilus galloprovincialis*) and Manila clam (*Ruditapes philippinarum*) will be presented, framed in the ecological context of the Northern Adriatic Sea and compared with ecologically different bivalve farming sites. Authors would stress that the evaluation of biocalcification contribution to carbon sequestration requires a site-specific approach, by means of combined *in situ* and remote environmental data collection.

Materials and methods

The carbon flows related to the bivalve biocalcification process were computed using data on the ratio of shell to total fresh weight and calcium carbonate content of mussels and clams specific to the study sites (Sacca di Goro, Emilia-Romagna, and Sacca di Scardovari, Veneto, Italy). The sites chosen are the most representative of bivalve production in Italy. Equation 1 was used for the calculation of CO_2 sequestered by the shell during the biocalcification process: (Eq. 1) CO_2 sequestered = mass $CaCO_{3\,shell}$ × CO_2 molecular mass / $CaCO_3$ molecular mass. Equation 2 was used for the calculation of the CO_2 released by the biocalcification process: (Eq. 2) CO_2 released = shell mass × Ψ × % $CaCO_{3\,Shell}$ × CO_2 molecular mass / $CaCO_3$ molecular mass, where Ψ indicates the ratio of CO_2 released per $CaCO_3$ precipitated as a function of seawater buffering capacity. Ψ for each site were calculated using the *seacarb* R package [1]. Environmental data required for Ψ calculations (site-specific pH, salinity, seawater temperature, and CO_2 partial pressure) and chlorophyll-a concentration for the study sites were retrieved from the AquaX platform (Colombosky, https://gis.aquaexploration.com), which collects data with a spatial resolution of 300 m^2 using Copernicus satellite data. Environmental parameters required for the mussel carbon flows computation were retrieved for two other, very different, farming sites, one located in Vigo (Spain) and one in the Osteerschelde National Park (The Netherlands).

A bibliographic review was conducted to collect supporting and conflicting evidence on the hypothesis that shell formation can represent a carbon sequestration mechanism.

Results and discussion

The environmental parameters at the four farming sites and the shell:total fresh weight ratio and carbon sequestration potential of mussels and clams are outlined in Table 1.

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	Sacca di Goro *(44.74, 12.29)*		*Sacca di Scardovari* *(44.88, 12.43)*		*Vigo* *(42.24, -8.80)*	*Osteerschelde National Park* *(51.54, 3.93)*
Chl-a (ug/l)	3.98		4.41		5.14	5.28
pCO_2 (µatm)	292.15		295.17		412.41	724.60
pH	8.17		8.16		8.02	7.73
salinity	32.75		35.55		33.13	13.33
T (°C)	18.20		17.20		15.41	12.96
Ψ	0.66		0.64		0.73	0.91
	Mussel	**Clam**	**Mussel**	**Clam**	**Mussel**	**Mussel**
%$CaCO_3$	90.7	93.4	91.5	93.8	91*	91*
CO_2 sequestered (kg CO_2/kg)	0.18	0.24	0.19	0.24	0.18	0.18
CO_2 released (kg CO_2/kg)	0.12	0.15	0.11	0.15	0.13	0.17
Net CO_2 flow (kg CO_2/kg)	**-0.06**	**-0.09**	**-0.08**	**-0.09**	**-0.05**	**-0.01**

**Data on % $CaCO_3$ and shell: total fresh weight ratio not available for the Spanish and Danish sites, the average values of the shell composition at the Italian farming sites have been used.*

Reviews on the possible contribution of bivalve farming in mitigating the effects of climate change are numerous [2]–[4]. However, the role of biocalcification as a carbon sink is controversial [5]–[7] and this debate arises when the ecological context is not properly considered. Two molecules of dissolved CO_2 (HCO_3^-) are required during shell formation. One precipitates into the shell, and the other is released into the seawater. This process leads to a decrease in Dissolved Inorganic Carbon concentration by one unit and a decrease of Total Alkalinity by two units. Consequently, the biocalcification process, in a closed and isolated system, leads to a lowering of pH and an increase in pCO_2, thus limiting the capacity of seawater to absorb atmospheric CO_2. In this context, the formation of the bivalve shell should not be defined as a carbon sink. However, if the biogenic calcification is framed in a coastal epipelagic and highly productive area, the CO_2 released from the biocalcification process is likely to be promptly taken up by photosynthetic organisms, according to the combined equation: $Ca_2+ + 2HCO_3^-$ □ $CaCO_3 + CH_2O + O_2$ [8]. Therefore, the raise of pCO_2 induced by the biocalcification process should not take place in those environments characterized by high primary production (Table 1). Hence, in this context, biogenic calcification can act as a potential carbon sink [6]–[8]. This conclusion is also supported by [9], who demonstrate the overall carbon sink behaviour of the North Adriatic Sea, although characterized by spatial and temporal variability. From the comparison among different farming sites, where different ecological parameters determined high variation in net CO_2 flow, it is evident the necessity to evaluate the carbon sink potential with a site-specific, local approach.

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ENVIRONMENTAL PERFORMANCE OF AN ITALIAN CLAM HATCHERY – A LIFE CYCLE ASSESSMENT APPROACH

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Introduction

The aquaculture sector has experienced important growth during the past decades to meet the seafood demand that fishing practices cannot always sustain. Shellfish represent more than 50% of the global aquaculture production and, in the past two decades, bivalve production increased by 82%, reaching 17.7 million t in 2020 [1]. Shellfish are an important component of diets all over the world for their high nutritional values. Furthermore, many studies have proved that bivalve aquaculture, if carried out in a sustainable way, could offer important ecosystem services (e.g., nutrients regulation, coastal protection, boosting biodiversity, carbon removal, and food provisioning) [reviewed in 2]. One of the most economically important farmed bivalves in Europe is the Manila clam Ruditapes philippinarum. In 2020, Manila clam aquaculture production in the EU-28 overreached 25,290 t, 96% of which was supplied by Italy [3]. Clam farming consists of the following phases: seed procuring and sowing, management of the farming area and clam density in the seabed, harvesting, and depuration. Clam seed is usually collected in natural nursery areas. However, during the past two decades, a serious decline in wild clam populations, and therefore spat availability, has been observed [4]. The causes behind this reduction are poorly understood, but, likely, they lie in phenomena acting synergistically, such as rising seawater temperature, anoxia, eutrophication, environmental quality degradation, and diseases. To cope with the shortage of natural seed, Italian clam farmers also rely on seed produced in hatchery facilities (most of which is imported from EU countries and the United States), where microalgae production, broodstock conditioning, spawning, and larval rearing (up to 6 mm) take place. Against the context of climate change and its effects, it is likely that, in the future, clam farmers will be more dependent on hatchery-produced seed. The environmental burdens associated with hatchery seed production are expected to be higher than wild seed procuring. This contribution reports the results of the first Life Cycle Assessment model applied to a Manila clam hatchery. The case study applies to the only hatchery present in Italy, which produces about 5 t of seed/year. The environmental impacts of hatchery seed production are quantified, and potential hotspots are identified, to provide useful suggestions to improve the environmental performance of the clam supply chain.

Materials and methods

The case study referred to a Manila clam hatchery (reference year: 2020) located on the North Adriatic Sea coast, Goro (FE, Italy), one of the most productive areas for clam farming. The LCA approach follows the guidelines of [5]. The objective of this study was to assess the environmental performance of the clam hatchery, by analyzing material and energy flows through the system. A cradle-to-gate analysis has been carried out, considering the following processes: 1) microalgae production, 2) broodstock maintenance and conditioning, and 3) larval rearing until the marketable size was reached. The system boundaries included the above processes and all material and energy inputs and outputs to and from the system. The functional unit (FU) chosen was 1 kg of live clam seed. The *Life Cycle Inventory* was based on data provided by the technical personnel of the hatchery through questionnaires and interviews (foreground data). The Ecoinvent 3 database has been used to gather data about the production of electricity and raw materials (background data). The *Life Cycle Impact Assessment* has been carried out using the software SimaPro 9.1.0.7 (PRé Consultants), adopting the ReCiPe 2016 (H) method.

*Table 1 Impact assessment of hatchery seed production– Characterization. Results refer to 1 kilogram of live clam seeds. *The Global Warming impact category does not consider the biogenic carbon flows related to shell formation.*

Midpoint Impact Category	Unit	
Global warming*	kg CO_2 eq	26.432
Terrestrial acidification	kg SO_2 eq	0.097
Freshwater eutrophication	kg P eq	0.009
Marine eutrophication	kg N eq	0.001
Fossil resource scarcity	kg oil eq	8.227
Water consumption	m^3	0.575

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Results and conclusions

The environmental performance of the hatchery seed production is reported in Table 1. The main driver of the environmental impacts was electricity consumption, with a contribution larger than 80% for the selected impact categories. Liquid oxygen consumption followed, with a contribution of 3-12% depending on the impact category considered.

It is worth pointing out that this work specifically computes the environmental impacts associated with the hatchery phase for clam seed production. Therefore, the FU chosen is 1 kg of live clam seed, which corresponds to about 20,000 individuals. Considering a minimum survival rate of 60% (data estimated by farmers) and a mean individual weight of 10 g for adult clams, 1 kg of clam seed would produce about 120 kg of commercial-sized clams.

The results confirm that electricity consumption, which partially depends on fossil fuels, is one of the biggest bottlenecks to the environmental sustainability of food supply chains. This agrees with the only other study documenting the environmental performances of an oyster hatchery in the United States [6]. The environmental burdens associated with the production of clam seed could be reduced by switching to alternative technologies to cope with the energy demands of the facility, such for example, investments in photovoltaic and wind energy production systems. The improvement of the clam supply chain's environmental performance should be combined with the development of other clam hatcheries in Italy. This would reduce the environmental costs associated with the import of clam seed from Spain, France, the Netherlands, and the United States.

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LIPID OXIDATIVE RESPONSE IN FILLET OF GILTHEAD SEABREAM FED ORGANIC AQUAFEED INCLUDING CRAYFISH MEAL RICH IN ASTAXANTHIN

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Introduction

Fish lipids are mainly characterized by polyunsaturated fatty acids (PUFAs), which are extremely sensitive to oxidative decay during storage. Lipid oxidation is one of the main factors responsible for the reduction of fillets' sensory quality (flavour, colour, and texture), both in refrigerated and frozen conditions [1]. Oxidation is also linked to the loss of functional properties and nutritional value of foods [2]. However, its adverse effects may be slowed down by adding several antioxidant compounds. Among them, astaxanthin, one of the most represented carotenoids in aquatic animals, has become of great interest due to its powerful antioxidant activity [3] and its role in increasing the shelf-life of fish fillets [4]. In recent years, innovative and under-exploited ingredients for aquaculture feed formulation have been investigated. In recent studies [5], [6], the meal from whole red swamp crayfish (*Procambarus clarkii* Girard, 1852), an invasive freshwater crustacean rich in lipids, proteins, and astaxanthin [7], has been included as a functional ingredient in diets for rainbow trout, gilthead seabream and red porgy [5], [6], [8] due to its nutritional properties and effects on skin/fillet colour [6], [9]. Given the rapid growth of organic aquaculture [10], demand for natural astaxanthin is rising for certified aquaculture feeds. However, natural astaxanthin sources are scarce, and their real effects are still poorly studied.

This work aimed at evaluating the effects of an organic aquafeed formulation including *P. clarkii* meal, on the shelf life and quality of gilthead seabream (*Sparus aurata*, L. 1758) fillets, previously refrigerated or frozen.

Materials and methods

A two-month feeding trial (July to September 2021) on *S. aurata* was carried out within the CREA permanent experimental device in Capraia Island (Livorno, Italy). The device consists of two commercial-sized mariculture cages, placed inside a local aquaculture facility. In the first phase, for one month, a total of 7,000 fish (3,500 per cage, mean weight of 400 g) were fed with commercial organic feed. During the second phase, the control cage (CTRL) continued to be supplied with the organic commercial diet, while the experimental cage (EXP) shifted to the organic feed including *P. clarkii* meal. The meal was obtained from about 400 kg of *P. clarkii* caught as part of the containment plan of the species (Legislative Decree 230/2017 of the Lazio Region) in the Nazzano Tevere-Farfa Regional Nature Reserve (Rome, Lazio), a wetland of high naturalistic value. After the crayfish were shredded and dried, 1,000 kg of 10% crayfish meal pelleted feed was produced (Nutri-Tech SRL, Mantova, Italy). The two diets were formulated to be isolipidic and isoproteic. At the end of the trial, 200 fish from each cage were collected. EXP and CTRL refrigerated samples (kept at 4°C) were analyzed 1 and 4 days (T1 and T4) after collection; EXP and CTRL frozen samples (kept at -20°C) were analyzed 31 and 34 days (T31 and T34) after collection. The analysis of the antioxidant power of *P. clarkii* meal, diets, and fillets was carried out by measuring the decay time of the radical DPPH (1,1-diphenyl-2-picril-hydrazyl) [11]. To estimate the lipid quality of fillets, the Flesh Lipid Quality (FLQ) index was calculated. The FLQ index correlates the amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with the amount of total fatty acids (FAs) [12]. To verify the aptitude of seafood to increase the risk of cardiovascular diseases related to the quality of lipids, a Thrombogenic Index (TI) was also determined [12]. The fillets' oxidation level was evaluated by measuring the levels of thiols and malondialdehyde (MDA), valuable indicators of meat degradation and oxidative stress [13]the redox balance can be affected by training regimen, dietary macronutrient ratio and their interaction. In this study, we conjointly evaluated the effects of physical activity (by voluntary swimming (VS. Finally, the increase in volatile nitrogen content (used as freshness index), mainly ammonia and trimethylamine (TMA), which are primarily responsible for the repulsive scent of fish, was measured [14].

Results and Discussion

Growth performances between the two cages were not significantly different. Concerning fillets nutritional quality only lipid content was significantly different between the two groups, being more abundant in the CTRL group (4.4% d.w.) than in the EXP group (3.6% d.w.) (ANOVA $p < 0.05$). The EXP fillets showed a fatty acid profile characterized by a low content of saturated (SFAs) and monounsaturated FAs (MUFAs), and high content in PUFAs, notoriously important in the human diet (especially omega 3). PUFAs are highly oxidizable components, causing faster oxidation of the flesh. As a result of the experimental diet, the presence of astaxanthin in the crayfish meal has counteracted this effect. Indeed, in the DPPH analysis, the higher the antioxidant capacity, the greater the scavenging activity [11]: crayfish meal has given excellent results (38.6% of scavenging activity), which are reflected in the EXP feed (34.5% of scavenging activity). CTRL feed showed the lowest values (1.1 % of scavenging activity). As for the fillets, the antioxidant power decreases over time, although the EXP fillets showed, on average, higher values for the entire duration of the storage trial. Significant differences (ANOVA $p < 0.05$) were found between the T1 and the frozen samples (T31, T34), for both CTRL and EXP. The EXP fillets showed a significantly higher FLQ index than CTRL (28.6% and 21.13%, respectively), indicating higher quality of lipids. The TI (which increases with the risk of blood clots [12]), was higher in the CTRL fillets compared to EXP ones, although remained both within the low-risk threshold (< 0.40). High values of thiols indicate less oxidation of the fillet [12]: the EXP fillets showed a significantly higher content of thiols than CTRL. Regarding MDA, EXP fillets showed lower values than CTRL, highlighting lower oxidative stress [12]. For the analysis of the freshness, the seabream fillets for the two treatments at T1 and T4 were evaluated: the analysed samples fell below the value of 9.3 mg / 100g of volatile nitrogen (which identifies very fresh fish), obtaining values of about 6 mg / 100g for the fillets in the two treatments at both storage times. No significant differences were found.

This study highlighted encouraging results regarding the use of red swamp crayfish as a natural and functional ingredient in diets formulated for organic gilthead seabream farming: (1) a growth comparable to the commercial diet, (2) high antioxidant power due to the presence of astaxanthin, (3) a lower sensibility to oxidation, and (4) a high content in PUFAs, especially omega 3.

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THE ROLE OF OLEIC ACID IN FAT DEPOSITION AND OXIDATIVE STRESS IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

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Introduction

The use of diets with high-lipid levels may lead to an increase in tissue fat deposition and susceptibility to lipid peroxidation, jeopardizing fillet quality, which is of major concern for the final consumer. To mitigate these potential harmful effects, several nutritional strategies have been applied, including the use of functional additives.

Oleic acid (OA) is a monounsaturated fatty acid (FA) that has been receiving special attention as a functional ingredient in mammal diets, due to its beneficial effects on weight gain and fat deposition control by interfering with feed intake (FI). Recent evidence in mammals also suggests that Oleoylethanolamide (OEA), a FA ethanolamide produced in the intestine from food-derived OA, and other N-acylethanolamines (NAEs) (e.g. anandamide, AEA; and palmitoylethanolamide, PEA), could be involved in FI regulation, possibly acting as satiety signal. Beside the impact of NAEs on FI regulation, other biological effects, including anti-inflammatory, analgesic and neuromodulatory effects were also reported (Diep et al., 2011).

In fish, OA dietary supplementation has also been associated with FI regulation (Librán-Pérez et al., 2014). Recently, we have seen in European seabass that supplementation of high-lipid diets with 1 and 2 % of OA reduced FI and enhanced feed efficiency without affecting growth performance (unpublished data). However, the interplay among FI, fat deposition, and lipid metabolism regulation by dietary OA in fish was not yet fully explored. Besides its role in FI regulation, in higher vertebrates, OA has also been described as an antioxidant and anti-inflammatory agent. In fish, however, the role of OA in the oxidative status and anti-inflammatory response is still scarcely studied. Thus, the present study aimed to evaluate in European seabass (*Dicentrarchus labrax*) fed high lipid diets the effect of dietary OA supplementation on fat deposition and its potential as an antioxidant. Taking in mind the growing importance of lipid-derived signals as feeding regulators in mammals and the lack of knowledge on the possible involvement of NAEs on FI regulation in fish, an additional aim of this study was to investigate the link between dietary fat content/OA levels and intestinal/tissue levels of NAEs.

Material and methods

Triplicate groups of 22 European seabass (Initial body weight of 21.4g) were fed six isoproteic diets (45% crude protein) containing two dietary lipids levels (16 and 22%) and supplemented with 0, 1, or 2% of OA (diets 16L:0OA; 16L:1OA; 16L:2OA; 22L:0OA; 22L:1OA; 22L:2OA). Fish were fed these diets twice a day to apparent visual satiation, six days a week, for 10 weeks. At the end of the growth trial, liver, hypothalamus, intestine, and muscle from 3 fish per tank were collected and stored at -80° C until enzymatic assays, and glutathione, OEA, AEA, and PEA quantification. Adipose tissue, muscle, and liver from another 3 fish per tank were sampled to assess fat deposition mechanisms.

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Results

At the end of the trial, OA supplementation increased the hepatic G6PDH but decreased malic enzyme activity and decreased mTOR expression. Hepatic Akt, phosphorylated Akt (p-Akt), and p-mTOR expression were not affected by the dietary treatments. The inclusion of OA in the diets did not affect OEA levels in the tissues analyzed but decreased intestinal AEA and PEA levels.

Regarding antioxidant enzymes, GPX, GR, and CAT were not affected by dietary treatments, while dietary lipid levels increased SOD activity in the intestine. The highest antioxidant enzymatic activity was observed in the liver and intestine compared to muscle. Intestinal SOD and GR activities were higher than in the liver and muscle.

Hepatic tGSH and GSH levels were higher than in the intestine and muscle, while GSSG level was higher in the intestine than in the liver and muscle.

Overall, the oxidative status, measured as OSI and LPO, was not affected by dietary treatments but was different in the tissues analyzed, with the highest OSI and LPO levels being observed in the muscle, followed by the intestine and liver.

Conclusions

The present results suggest that OA may affect lipid metabolism and fat deposition by decreasing mTOR expression, without affecting the phosphorylated forms of Akt and mTOR, suggesting that the fatty acid synthesis in the liver was not induced. Moreover, while dietary OA did not affect OEA levels in the tissues, it promoted a decreased AEA and PEA suggesting an enhancement of anti-inflammatory response. Dietary OA supplementation did not affect the oxidative status of European seabass.

Although dietary lipids and specific fatty acid as OA are major nutritional factors regulating OEA production in mammals, the role of dietary lipids/fatty acids in the regulation of European seabass OEA intestinal levels was not clarified as no changes on OEA content were observed. On the other hand, the decrease in AEA and PEA intestinal content of European seabass observed in response to dietary OA intake supports a regulatory role for these NAEs in FI control or other biological roles that need to be further investigated.

Acknowledgments

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ZEBRAFISH SCALES AN INDUCED-INFLAMMATORY MODEL FOR HIGH-THROUGHPUT SCREENING OF ANTI-INFLAMMATORY MOLECULES

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Introduction

Intensive fish production in aquaculture is a stressful environment that increases susceptibility to opportunistic pathogens and diseases, ultimately leading to fish death. This implies economic losses for producers and reduces the growth of a sector that is considered one of the major sources of dietary protein for human consumption[1]. To reduce the impact of opportunistic pathogens, rapid methods for the selection of novel anti-inflammatory and immunostimulants are needed to modulate and strengthen fish immunity and prevent/control disease outbreaks.

Zebrafish is a model of excellence to unravel the immune mechanisms and signaling pathways[2], due to its complete sequenced genome and advances in genomic engineering technologies. It has already been used to comprehend inflammatory disorders affecting fish and mammals due to the conservation of immune response mechanisms. In particular, the zebrafish scales have been used as a semi-high-throughput system for drug discovery in a variety of biomedical applications[3]. Moreover, scales have been suggested to be used as a screening platform to reduce the number of animals and as an alternative to *in-vivo* experimentation. Here we show that scales can be used as a powerful model for the study of the immune response to bacterial infections. We observed an increase in the number of macrophage cells (*mpeg1.1*$^+$ cells) responding to lipopolysaccharide stimulation, making scales a suitable and rapid methodology to identify novel inflammatory and immunostimulant molecules.

Materials and methods

Adult zebrafish (*Tg*(*mpeg1.1:eGFP*)^{gl22}, 3-6 months) were maintained in 3.5-L tanks in a 980-L recirculating aquaculture system (ZebTEC®, Tecniplast, Italy) with the following water parameters: temperature - 28 ± 0.5 ºC, conductivity - 750 ± 25 µS/cm, pH – 7.5 ± 0.2 and, photoperiod - 14:10 h light:dark. Water quality was maintained as described in Martins *et al*.[4]. Fish were maintained in a feeding regime using ZEBRAFEED® (400-600µm, Sparos Lda, Portugal).

Scales were plunked from fish anesthetized with MS-222 (Sigma-Aldrich, Spain). Thereafter, scales were washed with sterile PBS and cultured as described in Pasqualetti *et al*.[5]. Briefly, scales were cultured for 72 hours post-exposure (hpe) with L-15 media (Gibco™, Fisher Scientific, Portugal) containing L 10% fetal bovine serum, 1% L-glutamine, and penicillin-streptomycin. *E. coli* lipopolysaccharide (LPS, Sigma-Aldrich, Spain) stocks were prepared at 1 mg/mL, 10 mg/mL and 100 mg/mL, and used for the preparation of media containing 1 µg/mL, 10 µg/mL and 100 µg/mL, respectively. Culture media was replaced every 24 hpe. Scales were photographed at 24, 48 and 72 hpe with Axiocam 305 mono (Zeiss, Germany) coupled to a Zeiss SteREO Lumar V12, and images were processed using ImageJ software where the number of macrophages (*mpeg1.1*$^+$ cells) was counted.

Data were expressed as a box plot with median ± min/max. Normally distributed data (Shapiro-Wilk´s test) were analyzed with an unpaired t-test -Welch's correction ($P \leq 0.05$).

Results

Scales plunked from adult zebrafish (*Tg*(*mpeg1.1:eGFP*)^{gl22}, 3-6 months) and exposed to different concentrations of LPS (i.e., 1 µg/mL, 10 µg/mL, 100 µg/mL) in an *ex-vivo* culture system. We observed a significant increase in the number of macrophages during the first 24 hpe for the higher concentrations tested (10 µg/mL, 100 µg/mL). However, a reduction in the number of macrophages was observed for LPS 100 µg/mL at 48 and 72 hpe, relatively to 24 hpe, but this did not occur for LPS 10 µg/mL (Figure 1).

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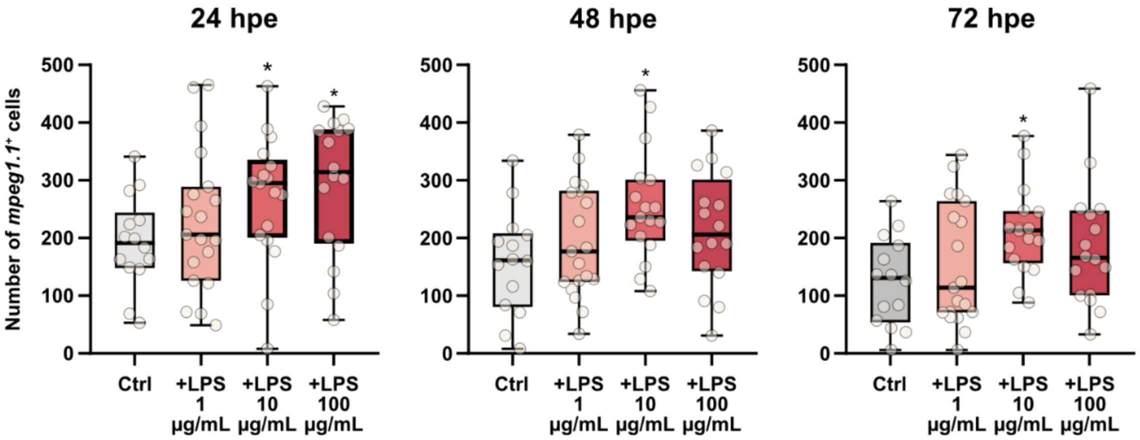

Figure 1. *Ex-vivo* exposure of zebrafish scales to LPS (1 µg/mL, 10 µg/mL, 100 µg/mL) for 72 hours post-exposure (hpe). Asterisks (*) represent significant differences among treatments (unpaired t-test with Welch's correction, $P<0.05$).

Discussion

Zebrafish scales have been used both as *in-vivo* and *ex-vivo* systems, for semi-high-throughput screening of compounds[3], and were addressed as a viable alternative method to the *in-vivo* culture systems. A recent study showed a unique characteristic of scales by their content in phagocytic macrophages (*mpeg1.1*$^+$ *cells)*[6]. However, limited data exist on their suitability as a model to uncover the mechanisms of inflammatory disorders. Our data revealed that occurs an increase in the number of macrophage cells in response to LPS stimulation, where a concentration of 10 μg/mL showed a significant increase in the number of macrophage cells (*mpeg1.1*$^+$ cells) at 24, 48, and 72 hpe compared to the Ctrl group. The *ex-vivo* culture of zebrafish scales can serve as a powerful model for the comprehension of fish immune response to bacterial infections and the search for novel anti-inflammatory molecules to be used for prophylaxis or treatment of infections in fish aquaculture. Its use in research settings allows for the decrease, repurpose, or reuse of animals respecting the 3Rs policy and is an ideal system to overcome the complete elimination of animals for research settings after 2030.

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Acknowledgments

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FIRST APPROACH TO THE USE OF *Ulva ohnoi* PRODUCED BY IMTA-RAS SYSTEMS AS FOOD INGREDIENT

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Introduction

Novel foodstuffs demand sustainable ingredients rich in micro and macronutrients and free of any toxic substances that might be in the environment. Seaweed and their derivates are widely used as ingredients in some food products worldwide as additives (alginate salts and carrageenan) or are directly consumed as *Ulva* spp. (Sea lettuce) in some countries. *Ulva* algae is rich in protein and minerals but there is scarce knowledge about their antioxidant activity and phytochemical composition (Vazquez-Rodriguez and Amaya-Guerra, 2016). Some research is actually focused in how the management of light in algae culture tanks might affect the yield and nutritional quality of the crop in IMTA-RAS systems (UlvaPRO Subproject 2). This work presents the preliminary results about the effect of different conditions of light management and preservation processes after harvest in the antioxidant capacity and bioactive compounds in *Ulva onhoi*.

Material and methods

The optimization of *Ulva*'s culture conditions, emphasizing on the management of light, is approached from a multidisciplinary perspective. *Ulva* was cultured in the circular tanks with different stocking densities and incident irradiances. *Ulva* fronds were washed and homogenized. Sample was divided in two portions. One fraction was dried by air at 60±2°C for 48 h, the other one was lyophilized during 48 h until constant weight.

After removing the moisture some parameters were determined: phenolic compounds content (by Folin-ciocalteu method) and antioxidant capacity (by ORAC assay, Cuprac assay and DPPH assay). Dried and lyophilized algae samples will be used directly as an ingredient in energy bars.

Results

The effect of light distribution in culture tank affected drastically the dry matter content of *Ulva onhoi* (Figure 1). Depending on the culture conditions, up to 4 % is found ($p<0.05$).

Results obtained will help to understand how the crop management may have an effect in the bioactive compounds of the algae and, additionally, the potential of the dried/lyophilized biomass as food ingredient.

Acknowledgments: Work funded by Spanish MICIU (RTI2018-095062-A-C22).

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UlvaPRO Subproject 2, https://futur.upc.edu/25161329?locale=en

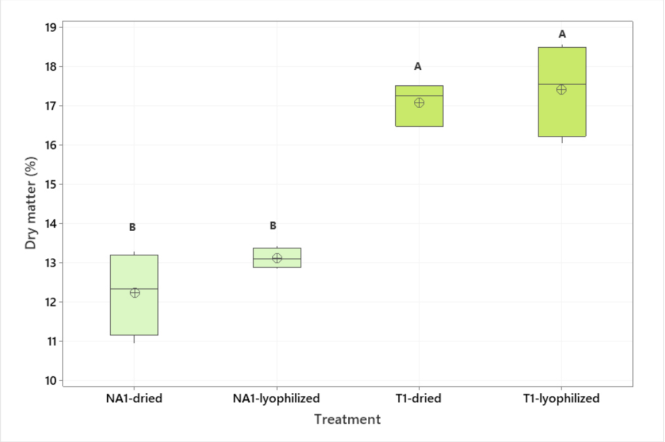

Fig 1. Dry matter content of *Ulva onhoi* depending on light distribution and presevation process. Different letters means significant differents between samples (Tukey, $p<0.05$).

LIGHT MANAGEMENT STRATEGIES TO MAXIMIZE *Ulva* PRODUCTIVITY IN IMTA-RAS SYSTEMS

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Introduction

The integration of fish farms with seaweed in recirculation systems is a promising line of action for the diversification and sustainability of aquaculture, and will contribute to the reduction, use and recovery of waste and by-products. The incident irradiance on the surface of the seaweed cultures, expressed as µmol of photosynthetic photons per square meter and second (μmol m^{-2} s^{-1}), is frequently the determining factor in the growth and photoinhibition processes, and one of the main challenges to increase the productivity and reduce the land surface required for seaweed production in the IMTA-RAS system.

The engineering of these IMTA-RAS systems has been studied (UlvaPRO Subproject 2, https://futur.upc.edu/25161329?locale=en), emphasizing the adequate management of light in algae culture tanks to obtain the best macroalgae production, both from the quantitative and qualitative point of view. Here we presented the main results obtained in an IMTA-RAS system (*S. senegalensis*-*Ulva ohnoi*). The studies conducted included:

-The distribution of light in seaweed tanks in function on irradiance, stocking densities and chlorophyll content of the *Ulva*;
-The effects of light distribution on growth and photoinhibition processes in *Ulva*;
-The effects of light distribution on nutritional quality and food safety of the *Ulva*.

Material and methods

The optimization of *Ulva*'s culture conditions, emphasizing on the management of light, is approached from a multidisciplinary perspective. According to the studies conducted, different experiments were carried out:

-Distribution of light in seaweed tanks:
Three circular tanks (64 cm diameter, 30 cm height) with bottom air injection (8 l min^{-1}) and opaque walls that received the irradiance through the water free surface were used. Different incident irradiances in the surface of the tanks were used with different stocking densities.

For each stocking density and incident irradiance, five PAR measurements were made every 5 cm from the surface to the deepest point of the tank (5, 10, 15, 20,and 25 cm from the tank surface) at three distances from the tank wall (8, 16 and 24 cm). PAR measurements were made with an ULM500 Light Meter & Data Logger connected to a US-SQS/L sensor (Walz, Germany) taking 5 PAR measurements per second during 60 seconds (n=300).

-Effects of light distribution on growth and photoinhibition:
Ulva was cultured in the circular tanks with different stocking densities and incident irradiances. Chlorophyll fluorescence parameters (PAM-2100, Walz, Germany), chlorophyll content (determined with a portable chlorophyll optical meter using the method described by Masaló and Oca (2020)) and specific growth rates were determined along time.

-Effects of light distribution on nutritional quality and food safety:
Initial and final *Ulva* fronds in each combination of stocking density and incident irradiance used to study the effects of light distribution on growth and photoinhibition were washed, dried and homogenized. With these dried samples different parameters were determined: basic nutritional parameters (mineral content, proteins, phosphorus, macro and micronutrients; and also the nitrate content), dietary fiber content (soluble and insoluble), heavy metals and polyphenols.

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Results ***(to be updated for the final abstract)***
-Distribution of light in seaweed tanks:

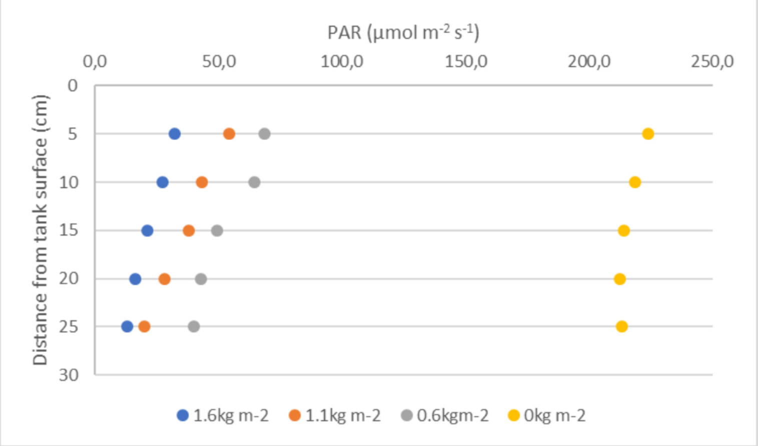

Fig 1. Example of light distribution at 24 cm from the tank wall in a tank with an avarege irradiance at tank surface of 250 µmol m^{-2} s^{-1}. Light measurements were made with different stocking densities (0.6, 1.1 and 1.6 kg m^{-2}).

-Effects of light distribution on growth and photoinhibition:
Experiments are currently carried out; results will be updated for the final abstract.

-Effects of light distribution on nutritional qualitity and food safety:
Ulva fronds are currently being stored for their posterior analysis; results will be updated for the final abstract.

Results obtained will help to identify the best algae management conditions for the most efficient use of light in order to maximize the productivity of the algae.

At the same time, the potential improvement of safety and nutritional quality of the algae produced in these systems will be assessed.

Acknowledgments: Work funded by Spanish MICIU (RTI2018-095062-A-C22).

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ROAD MAPS TOWARDS COASTAL-RURAL SYNERGY- THE COASTAL KNOWLEDGE EXCHANGE PLATFORM

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Introduction

The COASTAL project is funded by the H2020 Research and Innovation Programme[1] and aimed at improved coastal-rural synergy. The unique combination of systems modelling and multi-actor approaches with coastal and rural business entrepreneurs, administrations, stakeholders, and natural and social science experts resulted in evidence-based business solutions and policy recommendations, supporting rural and coastal development while preserving the environment. After four years of work with coastal communities, COASTAL is launching the **Knowledge Exchange Platform,** a **toolbox of resources** for boosting the coastal-rural synergy that provides the **space for stakeholders, experts and decision makers to interact, share and co-create new knowledge**.

The main goal of the KEP is to **direct target audiences to the information, data and tools, at a level of detail best suited to their interest.** All information, tools and services will be tailored to the needs of each audience type.

Objectives of the KEP

The specific aims of the KEP are to:

- Create **stakeholder awareness** of the case studies and the project as a whole;
- **Disseminate results**;
- **Utilise synergies** with, or build on, other existing knowledge sharing platforms;
- **Develop networks of practitioners** in relevant sectors;
- Create the basis for a **durable legacy of project outcomes**;
- Disseminate success stories of achievements of COASTAL to **promote the adoption of approaches to sharing knowledge**, add value, lever resources and **promote systemic innovations between experts and stakeholder communities** in the sectors relevant to coastal rural development.

Results

The KEP will provide target audiences tailored pathways for accessing project results and ensure best opportunities for further uptake and exploitation. When accessing it through the address https://h2020-coastal.eu/platform, the user will find a main menu consisting of six paths, providing further different types of content and presented in different formats as specified below.

1. **Best Practices & Policy Guidelines**
    a. Best Practices: It will consist of an interactive EU-wide inventory and qualitative analysis of best practices, business opportunities and innovative solutions related to coastal-rural collaborations.
    b. Policy Guidelines: A downloadable set of recommendations for improving the coastal-rural synergy from a policy perspective both locally and at an EU-level.
2. **Road Maps for Land-Sea Synergy**: interactive roadmaps are the compilation of all work carried out in each Multi-Actor Lab. These provide the context and bring business solutions and policy recommendations for the specific problems studied in each case study area, highlighting the actors involved, key resources need, beneficiaries and potential impacts at short-, mid- and long-term.
3. **Models for evidence-based policy analysis**: We are making available models developed during the project through a System Dynamics Modelling approach, for users to download them and adapt them to their specific conditions.
4. **Scenarios and Visions:** This section will present interactive flipbooks for the general public to read through. With visual content and simple language, we expect these flipbooks will give a good overview of what was done and achieved in each case study.
5. **Repository:** This section will store the Practice Abstracts developed for EPI-AGRI, summarizing in one paragraph each action, as well as all past newsletter issues published.
6. **User Forum:** This will be a space for stakeholders to interact, share knowledge, exchange ideas, discuss and collaborate on improving the synergies between rural and coastal environments. Each COASTAL MAL leader will be in charge of moderating the forum for each region and of boosting the users' participation.

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Conclusion

The Knowledge Exchange Platform is a platform for continued knowledge sharing, capacity building and co-creation, aiming at giving coastal and rural operators and planning agencies access to knowledge, solutions, and experiences of other regions and operators facing similar problems around land-sea synergies. The use of its resources would strengthen the opportunities for coastal and rural development and will facilitate land-sea collaboration across Europe.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N° 773782. https://h2020-coastal.eu

ASSESSMENT OF ENVIRONMENTAL MONITORING METHODS FOR INTEGRATED MANAGEMENT OF AQUACULTURE IN OPEN SEA: A MASS BALANCE MODEL APPROACH

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Introduction

Integrated Multi-Trophic Aquaculture (IMTA) appears to be an interesting alternative for the sustainable development of aquaculture activities. Based on the association of different species with trophic interactions, this concept aims to reduce the environmental impact of aquaculture and through this diversification would ensure more stable income for producers. The development of IMTA in coastal areas is promising (Chopin *et al.*, 2012; Buck *et al.*, 2018), but requires detailed documentation on trophic interactions between the various aquaculture activities. A multispecies activity can be defined as IMTA only once trophic interactions has been proven. Therefore, our capacities to describe trophic interactions is crucial and requires qualitative and quantitative methods.

Materials & methods

To address this issue, we have selected relevant tools to describe open sea IMTA. Indirect methods through the application of mass-balance modelling is one of those. By quantifying nutrients exchanges (*i.e.,* nitrogen and phosphorus fluxes) between the aquaculture compartments and with the nearby environment, this modelling approach aims to assess the impact of nutrient inputs coming from salmonid farms toward others aquaculture extractive compartments and may help the management of aquaculture activities. This approach allows also to estimate consequences of increasing the production of one or more aquaculture activities.

We evaluate this approach in the Trieux estuary (Côtes d'Armor, France) where coexist salmonid farms, oyster, mussel, and seaweed productions. In details, survey among professional producers were firstly conducted to collect input data for modelling (*i.e.* period of production, initial and final biomass produced, mortality, quantity of feed provided by fish farmers, etc.). Then, growth and mass balanced models were applied for each aquaculture compartment: the fish compartment (*Oncorhynchus mykiss*), the shellfish compartment (*Crassostrea gigas*) and the seaweed compartment (*Alaria esculenta),* during 8 months, from November 2021 to June 2022, corresponding to the period production of fish and seaweed compartments. Finally, a global mass-balance model including nutrient inputs from the watershed catchment area was produced with and without effluents from the fish compartment.

Results & Discussion

Application of the various mass balance models allowed us to estimate N and P from each compartment. We calculated that trout emitted 55.2 g N/kg and 10 g P/kg of fish, oysters emitted 220 mg N/kg and 70 mg P/kg of oyster meat, and algae absorbed 4.15 g N/kg and 0.68 g P/kg of algae relating to the entire biomass produced over the study period. Still, the most important thing to remember is that over the study period, the fish farming compartment emitted 357 kg N and 79 kg P, while the oyster farming compartment can be considered as a good extractor since it removes 4,636 kg N and 51 kg P, as well as the algal farming compartment, which removes 12 kg N and 2 kg P. The latest is of little importance because of the low tonnage produced. However, the quantity of N and P fixing rate by the oyster farming compartment must be qualified since the quantity of N and P ingested is not known. Then, if we consider only these three compartments that represent an IMTA system, the final N balance is negative, *i.e.*, there is more extraction of nutrients than emission into the environment. This is due to the oyster farming, which extracts a lot of nutrients. This result is in line with the interest in developing IMTA since nutrients are captured and extracted from the ecosystem. The final P balance is positive because the extraction by oysters is not sufficient to capture all the emissions of the fish compartment. However, when we take into consideration the nutrient flows from the watershed, the situation is quite different, the N and P balance is largely positive. Indeed, the watershed provides 99.75% of the N and 97.92% of the P of the estuarine ecosystem. The discharge of the fish farm represents only 0.08% of the N and 0.65% of the P, that goes through the estuary on a yearly basis, which is highly negligible.

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To go further, the final objective of such a project would be to integrate spatiotemporal variations in material flows. For this, using a direct method, via the monitoring of different trophic markers such as stable isotopes, fatty acids and/or trout DNA, would allow to validate the hypotheses posed by the mass balances. This is what has been done in the project, sampling of wild and farmed species has been carried out, the analyses are yet to be performed. Concerning the model part, it would be interesting to continue the project by setting up more precise production monitoring while developing other more complex models at different scales (ex. DEB, DEPOMOD, ECOPATH). These models would make it possible to conceptualize in a more exhaustive way all the flows of matter, from the species to the ecosystem by also modelling the flows related to the sedimented particles, and their hydrodynamics. Indeed, the distance between the aquaculture compartments is not considered at all here. However, the distance closely influences the capture of particles since the dispersion is determined by the hydrodynamics of the area. Thus, it is possible that the oyster and algal compartments do not capture, or capture very little, of the particles released by the fish compartment.

To sum up, this preliminary study applied an environmental monitoring methodology but did not allow for the identification of all material flows. The models applied are simplified and do not consider many flows such as flows between compartments, flows related to primary producers, or benthic flows. Moreover, the models do not allow to know if there is recycling of fish farming discharge outside the aquaculture compartments since the biocenosis compartment has not been modelled. Nevertheless, the study allowed us to determine the obstacles and levers that could be used to improve the method and increase the accuracy of the monitoring of material flows in estuarine IMTA systems.

Finally, this project addresses the complexity of the functioning of systems considered as IMTA in open sea, given the hydrodynamics of the environment and the nutrient inputs from the watershed.

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SEASONAL DYNAMICS IN CLEARANCE RATE OF THE JAPONESE OYSTER *Magalana gigas* REARED IN A BIVALVE NURSERY, RIA FORMOSA LAGOON, SOUTH OF PORTUGAL

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Introduction

The clearance rate (CR), defined as the volume of water cleared of particles per unit time, can reflect the physiology status of filter-feeding animals. This is one of the most important ecophysiological parameters for assessing growth, carrying capacity and influence of bivalves on ecosystem dynamics (*e.g.* Yu et al., 2010). CR is strongly dependent on endogenous factors such as: size, weight and reproductive cycles, as well as exogenous factors, which mainly include temperature, salinity, fluid dynamics and seston properties. Interpreting the CR results is crucial to understand how these factors can affect bivalve's filtration and consequently optimizing production systems. The most bivalve CR has been assessed under laboratory conditions, however this estimation does not generally provide a real information of what is found in the field (*e.g.* Clavier & Chauvaud, 2010). It has been found that the biodeposition method can provide continuous recording under field conditions and reflects the physiological status of bivalves more accurately than other methods (Yu et al., 2017). This method is uniquely suited for *in situ* experiments and can provide well founded CR data. In this study, we estimated the CRs of *Magallana gigas* juveniles of a bivalve nursery (Bivalvia), in the Ria Formosa lagoon, south of Portugal, using the *in situ* biodeposition method.

Material and methods

The experiments were carried out during Spring, Summer and Autumn seasons. The juveniles of oysters were acclimated in a suspended tank at a depth of ~0.5 m in the nursery Bivalvia (south of Portugal) for at least 2-3 days prior to the experiment. After acclimation, 500 oysters spat (length – 7±0.8 mm; wet weight – 0.11±0.05 g) were randomly placed in five cylindrical sediment traps for the CR measurements. Also, another five traps holding only oyster's shells were used as a control, to provided references for natural sedimentation. The cylindrical sediment traps were made of PVC, 1.2 cm holding mesh was hung 5.0 cm from the trap top and the top of each trap was then covered with a net of 1 cm mesh size. All sediment traps were randomly fastened to suspended tanks, in the middle of production system, at a depth of ~0.5 m. The environmental parameters (water temperature, pH, dissolved oxygen and salinity) were measured at the experimental depth (0.5 m) at the beginning and the end of the experiment. Triplicate water samples were collected to determine chlorophyll *a*, nutrients, total particulate matter (TPM), particulate inorganic matter (PIM) and particulate organic matter (POM). The individual CR (L ind^{-1} h^{-1}) was estimated using the biodeposition method (*e.g.* Sroczyńska et al., 2012): CR = (W – W0) C N T, where W is the inorganic matter weight from a trap with oyster (mg), W0 is the average inorganic matter weight from the control traps (mg), C is the average PIM concentration in the ambient water during each experimental period (mg L^{-1}), N is the number of oysters in the trap and T is the length of deployment period (h).

Results and discussion

Food availability is one of the limiting factors for the success of shellfish production. The effects of food competition resulting from spatial aggregation and by the high density of bivalve's production have been demonstrated in many situations and for different species. The availability and quality of food is usually the determining factor in growth models used to evaluate the success of a bivalve's production, namely in nursery phase. The CR of the juvenile oyster was higher during the spring trials (19.9 L g^{-1} h^{-1}) and lowest in the winter (11.5 L g^{-1} h^{-1}). This trend was also observed in food availability (chlorophyll *a*) and in TPM. Several studies have shown that the clearance rate increases with seston concentration up to a threshold beyond which this rate begins to decrease. This limit is species specific and for the oyster *M. gigas*, Barillé & Prou, (1994) considered that the CR increases up to a limit of approximately 50 mg L^{-1} of PTM. In the trials carried out in the Bivalvia nursery system, the maximum TPM values were recorded in the summer (25.80±8.77 mg L^{-1}), not exceeding this maximum. A holistic approach of all determined parameters showed that: a clear positive relationship between CR and food availability; seston did not affected the oysters feeding and consequently growth and the physical, chemical and biological characteristic of nursery seawater was not limiting for the good performance of juveniles. The results obtained contribute to the knowledge of the physiological behaviour of *M. gigas* juveniles in a nursery and constitute an important tool for application in energetic models of the species.

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Acknowledgements
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PRELIMINARY RESULTS OF CATCH SELECTION OF SMALL PELAGIC FISH IN ADRIATIC SEA: AGE AND MATURITY STAGE OF SARDINE *Sardina pilchardus*

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Introduction

The sardine (*Sardina pilchardus*) is a multiple spawner of indeterminate fecundity, belonging to the family Clupeidae and distributed throughout the Mediterranean. Sardine has separate sexes and sexual maturity is generally reached at the end of the 1st year at a length of 10 cm. (Ganias et al., 2004). In the Adriatic Sea, the spawning season lasts from fall to spring with a peak in winter. Sardines are among the most ecologically and economically important marine fish species and are subject to high fishing pressure worldwide (Domenella et al., 2016). Overfishing is undoubtedly the greatest threat to marine biodiversity and fish stock conservation. Global fisheries bycatch, the incidental catch of unexploited or unmanaged species, is also a major threat to the world's oceans. Otoliths are traditionally used to obtain information on the species, age, and size of fish (Rodríguez Mendoza, 2006), while histology is routinely used for sex determination, gonadal developmental stage, etc. (Blazer, 2002). Due to the low selectivity of fishing gear, fishermen catch sardines that are at different stages of development. The research proposes the integration of a live fish sorting device that will allow a relevant proportion of undersized fish that currently die during hauling to survive healthy and return to the sea. Adjustment of fishing gear and appropriate sardine catch size selection methods could reduce the proportion of juveniles of these species in the catch.

Material and methods

On a fishing vessel, the diameter of the grid (sea separator) was set (Ø=11 mm) as part of the vacuum pump fishing device (FAIVRE 8, France) used to transfer fish from the purse seine net into isothermal fish boxes. Fishing with the surrounding purse seine net was performed and the percentage of target species of the selected fish (N=100) caught in the central Adriatic Sea in March 2022 was recorded. The selected sardines were stored by freezing (-18°C) and then processed in the laboratory. By macroscopic and microscopic examination of gonads, gametogenesis was determined ICES (2008). Routine histology was used to prepare tissue sections for histological analysis. After fixation in Davidson fixative, tissues were further dehydrated with increasing alcohol concentrations, cleared in xylene, and soaked in paraffin wax using an automated Excelsior AS processor (Thermo Fisher Scientific Inc., USA). Paraffin block sections were cut into 2 µm sections using a Microm HM 355S microtome (Thermo Fisher Scientific Inc., USA). Tissue sections were stained with modified Harris hematoxylin and Young's eosin (Thermo Fisher Scientific Inc., USA) for histopathological evaluation and with periodic acid (Biognost, Croatia) for visualization of mucosal substance. Photomicrographs were taken with Axio Scan.Z1 scanning light microscope (Zeiss, Germany) and processed with image processing software ZEN 2.3. Age was determined by the number of microincrements on the sagittal otoliths (ICES, 2017). The extraction of otoliths (40) was performed in the laboratory. After extraction, the otoliths were cleaned in fresh water and stored dry in vials until age determination. Age determination was performed using a Dino-Lite digital microscope (Dino-Lite Europe, The Netherlands). Otoliths were immersed in 70% alcohol in a black petri dish and illuminated with light.

Results and discussion

The results of histological sections of the gonads of selected sardines indicated fish at different stages of gonadal development. Fig. 1 shows the female at the mature stage and the male at the early mature stage with central spermatogonia lobules (SG) filled with spermatids and spermatozoa (ST /SZ) and surrounded by a basement membrane (arrow). Primary oocytes of the female (PO), yolk vesicle stage oocytes (YV), primary yolk globule stage (PYG), secondary yolk globule stage (SYG), tertiary yolk globule stage, hydrated oocyte stage (HYD) with large oil drop (OD), postovulatory follicle (POF) (marked). The male was in the early maturation stage with central spermatogonia (SG) filled with spermatids and spermatozoa (ST /SZ) and surrounded by basement membrane (arrow).

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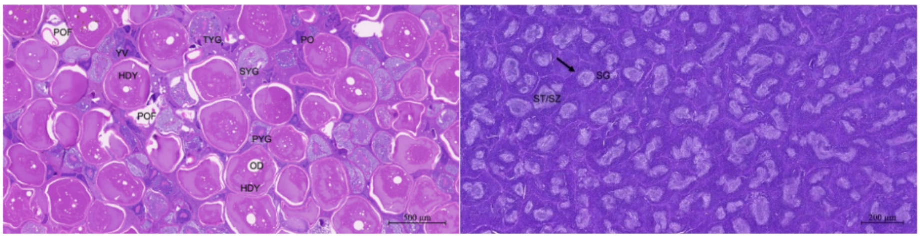

Fig.1. Histological sections of selected sardine ovary (left) and testis (right)

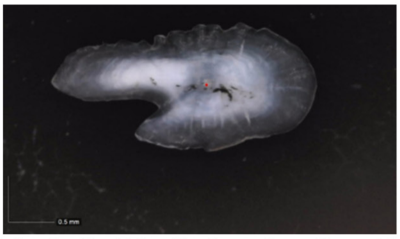

Fig. 2. Otolith of selected sardines (*Sardina pilchardus*)

Following the two-step procedure of counting anullus formation and applying the conventional rule of date of birth, most of the selected sardine samples were assigned to age group 1 (Fig. 2), with the exception of one individual that was assigned to age group 2.

Conclusions

The selected fish were at different stages of gonadal maturity and were assigned to age group 1. It is recommended that the small pelagic fish selector be further developed to improve its performance for even better selection of undersized individuals.

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PROBLEMS WITH SURVIVAL OF JUVENILE STURGEON IN CLOSED RECIRCULATION SYSTEMS AND WAYS OF SOLVING THEM

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Introduction

From an economic point of view, sturgeonsare of great value as a source of caviar and meat production. Over the years, populations of wild sturgeon have significantly declined due to overfishing and poaching, combined with water pollution and loss of natural habitats. This has led to the current status of the sturgeon family as one of the most endangered species among all animal species [1]. Recirculation aquatic systems (RAS) are considered to be the most environmentally friendly way of producing fish at a commercially profitable level, and sturgeon is one of the species of interest for rearing in RAS conditions [2]. RAS have been already successfully used in fish farms designed for sturgeon breeding (e.g. Russian sturgeon (*Acipenser gueldenstaedtii*), Baltic sturgeon (*Acipenser sturio* L.), sterlet (*Acipenser ruthenus*), beluga (*Huso huso*) for reproduction both in Ukraine and Lithuania. The rearing of sturgeon fish in RAS is accompanied by a number of problems caused by the intensification of the process. As a consequence, a decrease in nonspecific immunoresistance entails a number of illnesses caused by opportunistic microflora. Sturgeon juveniles are particularly sensitive to stress during periods of metabolic restructuring, which is clearly visible in the digestive system. It is known that in the early larval stages, there is a change in the secretion of digestive enzymes during the staged development of the digestive tract. P. Budington has identified three critical stages in the development of the digestive system of sturgeons, when they are particularly sensitive to unfavourable rearing conditions: The first stage (from 3 to 14 days after hatching) is the beginning of endogenous nutrition of pre-larvae (yolk sac) stage, the second (from 7 to 14 days after hatching) is the active nutrition stage, and the third stage (from 24 to 30 days after hatching) is metamorphosis, when the larval digestive system acquires functions typical of adults [3]. The transition to each stage is accompanied by increased mortality of sturgeon larvae. Probiotic preparations are often used to prevent this [4, 5].

The aim of our work was to study the effect of a complex probiotic preparation produced in Ukraine - “Emprobio” – on the survival of juvenile sturgeons during their rearing in closed systems.

Materials and methods

The experiments were carried out in RAS on small sterlet fry larvae, immediately after their transition to exogenous nutrition and lasted for 28 days at a temperature of 18°C. During the experiment a probiotic “Emprobio” was used, whichenriched feed organisms (*Artemia salina* L.). The duration of enrichment was 6 hours at a temperature of 5°C. The sterlet larvae were divided into three groups: 1000 specimens in each group according to the amount of probiotic in the feed : -0.4 cm^3/dm^3 -4 cc/dm^3 and a control group of sterlets received nauplii, probiotic. The fish were fed 6 times a day at a regular intervals. Food rationing was carried out taking into account daily requirement in live feed for sterlet larvae and fry which is 60 – 70% of fish body weight. Probiotic “Emprobio” is non-spore, liquid veterinary probiotic, it contains mixture of various live cultures, including bacterial cells *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*. Number of live microbial cells in $1cm^3$ of probiotic is at least 10^6-10^8. Bacterial inoculations were carried out using standard microbiological methods followed by bacterial identification using biochemical methods.

Results

Feeding with probiotic-enriched *Artemia* improves the growth processes which become visible after fourteen days of feeding. The average mass of the larvae in the experiments is 25.9 and 27.9% higher compared to the control group, respectively. At the same time, it is shown that there is no significant difference between the weights of fish which were enriched with different concentrations of microorganisms in the incubation mixture. Apparently, 0.4 ml of probiotic ($4x10^8$ cells/g live nauplia) is sufficient to induce a maximum release of microorganisms in the intestines, which provides a positive effect of beneficial microflora. During microbiological examinations of the intestines of juvenile sturgeons the opportunistic bacteria of the genus *Aeromonas*, *Pseudomonas* as well as enterobacteria were detected and identified. The positive effect of the probiotic “Emprobio” application is noticeable on the overall survival rate of fish already after the

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first day of use of the probiotic, when in the control group of fish 24,1 % of individuals died due to intestinal bloating. The survival rate after seven days of feeding with the probiotic in the experimental group No 1 was higher compared to the experimental group No 2 by 5 % with a probability 0.05. During the next fourteen days of the experiment the cumulative survival rate of fish in both experimental groups did not change, while in the control group gradually decreased to 51 %. The results of bacterial seeding of intestinal contents of sterlet juveniles from experimental and control groups confirmed the efficiency of the transfer. Thus, all lactic acid bacteria contained in the probiotic were isolated in the intestines of the experimental groups in the amount of 1×10^5 cells/g, as well as yeasts *Saccharomyces cerevisiae* in the amount of 3.2×10^2 cells/g. In the control group, there were only isolated colonies of lactic acid bacteria.

Conclusions

The rearing of larvae and their transition to artificial feed, as well as the rearing of early fry are the most complex stages in the technological cycle of sturgeon rearing in RAS. Survival, growth rate of commercial fish, feed utilization efficiency, and, consequently, the efficiency of the entire production depends on the quality of the produced juveniles. The search for and development of ways to apply new, safe and effective preparations that have a positive effects on the survival of juvenile sturgeon are one of the promising areas of research, and our work will have its continuation and application to other species of sturgeon in the future.

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THE SALTBOX PROJECT: WEIGHT-MORPHOMETRICS RELATIONSHIPS FOR INTENSIVELY REARED GILTHEAD SEABREAM *Sparus aurata*

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Introduction

Within the scope of the SaltBox project, i.e. the design and manufacture of a fish size/biomass estimator for gilthead seabream, it is necessary to establish accurate weight-morphometrics relationships, through which the image captured by the estimator can be "translated" to actual body weight. The aim of the present study is to report these relationships for gilthead seabream.

Materials and Methods

A total of 3312 specimens were individually weighed and photographed with embedded ruler. Targeted body weight ranged from 50 to 1000 g and special attention was paid to obtain at least 75-100 observations in a range of 25 g weight classes. Photographs were analyzed by digital image analyses and several morphometric traits were obtained. Pearson product-moment correlations were calculated, regression analysis was performed, and several models were applied to identify the most accurate one predicting weight from one or more of the morphometrics traits.

Results

The strongest correlation was obtained between weight and body monolateral area, followed by body lengths (i.e. total, fork, standards length, body height). The best model to describe weight (W)-morphometric traits (MT) relationships was of the type: W = aMTb, with the exception of W-eye diameter and W-eye area relationships, which were neither strong nor well fitted. Multiple or polynomial regression also produced inferior estimations. Also, when data were analyzed separately for body weight ranges of 50-100 g, 100-500 g and 500-1000 g, relationships obtained were weaker and did not offer any advantage over those of the full body weight range examined.

Conclusion

Results obtained indicate that body monolateral area can be best used to accurately estimate actual body weight. The size/ biomass estimator resulting from the SaltBox project can be tuned towards the precise estimation of this morphometric trait.

Aknowledgements

The project is co-funded by the European Maritime and Fisheries Fund and the Greek government.

EXTRUSION PROCESSING OF FISH FEED PELLETS: INFLUENCE OF SILICA AND OIL CONTENT ON THE PRODUCT PROPERTIES

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Introduction

Aquaculture is a fast growing market and there is a large demand for high fat fish feed especially for species like salmon and trout. The current production process for fish feed cannot introduce enough oil into the extrusion process because of the negative effect on the extrusion process, pellet stability, fat retention and nutritional quality (Huber, 2000). Therefore the standard production process includes a coating step after extrusion to increase the fat content which increases the production costs. This project aims to increase the fat content during the extrusion process by the addition of silica to the fish feed formulation.

Materials and methods

A fish feed formulation consisting of soy protein concentrate, wheat flour, fishmeal, and canola oil was processed in a lab-scale twin-screw extruder (TwinLab F 20/40; Brabender GmbH & Co. KG, Germany). The formulation without and with the addition of 1% of different silica grades (Aerosil® 200 F, ZeoFree® 600, Sipernat® 50, Evonik Operations GmbH) was feed into the 0D opening. Water was added at 10D and canola oil was added at 20D to the extrusion process to increase the fat content up to 37% (Fig. 1). The resulting fish feed pellets were analyzed for abrasion resistance and fat loss.

A stable extrusion process for fish feed pellets was established with fat contents of 22, 27, 32 and 37 percent. 1 % of silica in the formulation reduces the lubricating effect induced by the addition of oil (Fig. 2). It also reduced the viscosity and thereby the thermo-mechanical energy input resulting in enhanced product quality. Depending on the silica grade the resulting pellets showed a 40 to 70 % higher resistance to abrasion compared to the pellets without silica (Fig. 3). It also reduce fat leakage of the pellets by 10 to 50 percent compared to samples without silica.

Conclusions

With the addition of silica it was possible add a high amount of oil and to establish a stable extrusion process without the need for an additional coating step after extrusion. The addition of silica reduces the lubricating effect of the addition of oil in the extrusion process and generates fish feed pellets with higher abrasion resistance and lower fat leakage.

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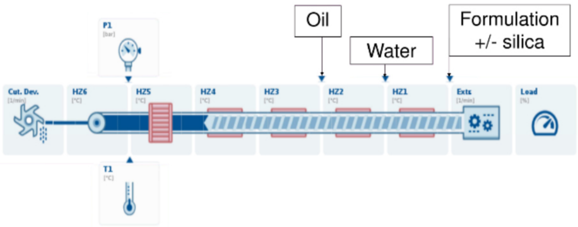

Fig. 1: Extrusion process with feeding positions of formulation, water and oil

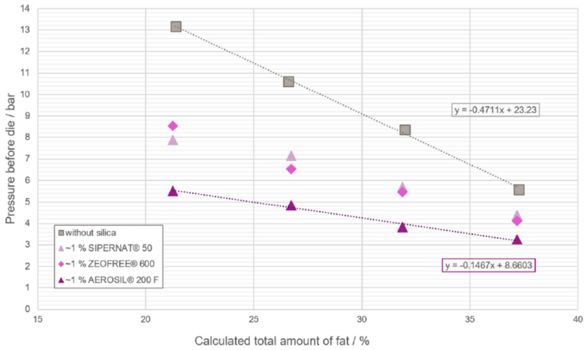

Fig. 2: Pressure at the extruder die for different amounts of oil addition with and without silica

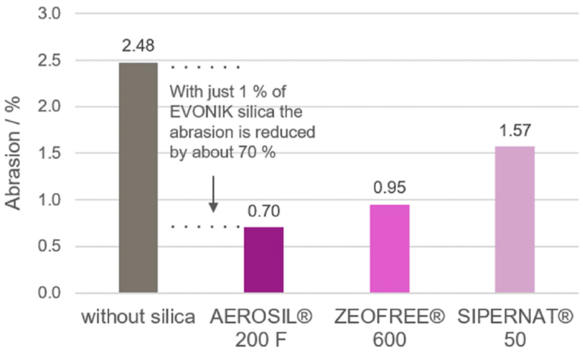

Fig. 3: Abrasion of fish feed pellets with 32% fat content without silica and with the addition of 1% of different silica grades

CLIMATE PROJECTIONS AND SEASONAL FORECASTING OF THE OCEAN

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Introduction

Seasonal forecasts and climate projections are used in a wide range of sectors and applications, from tourism to energy generation and food production. They provide opportunities for evidence-based long-term planning and early warning of unusual conditions, supporting an ecosystem-based management approach and helping in the design and implementation of nature-based solutions. Predictions of the ocean state, particularly at this time scale, have the potential to aid management of marine resources and to ensure effective protection of our marine environment.

Materials & Methods

The Centro Euro-Mediterraneo sui Cambiamenti Climatici (CMCC) produces a range of ocean-based products, including seasonal forecasts and longer-term climate projections. As an operational seasonal forecasting centre, we provide freely-available 6-month forecasts of physical atmospheric and oceanic variables via the Copernicus Climate Change Service. Here, we highlight the skill of our system to detect surface and subsurface marine heat waves (MHWs) and cold spells (CSs) in European seas.

Results

In collaboration with ISPRA (Italian Institute for Environmental Protection and Research), we have identified examples of mass-mortality events which have affected the aquaculture industry. For instance, there was mass mortality of farmed mussels in the Gulf of Taranto in summer 2015 due to several weeks of marine heat wave conditions. Forecasts of ocean conditions during these events are encouraging (Figure 1), and collaborative efforts with aquaculture stakeholders are necessary to fully understand the reliability and use of forecasts. As a result, we are actively looking for collaborations to co-develop useful marine indicators based on our products. To help us understand aquaculture stakeholder needs, attendees are invited to fill in our survey *“Climate projections and seasonal forecasting for aquaculture”* (see link in poster).

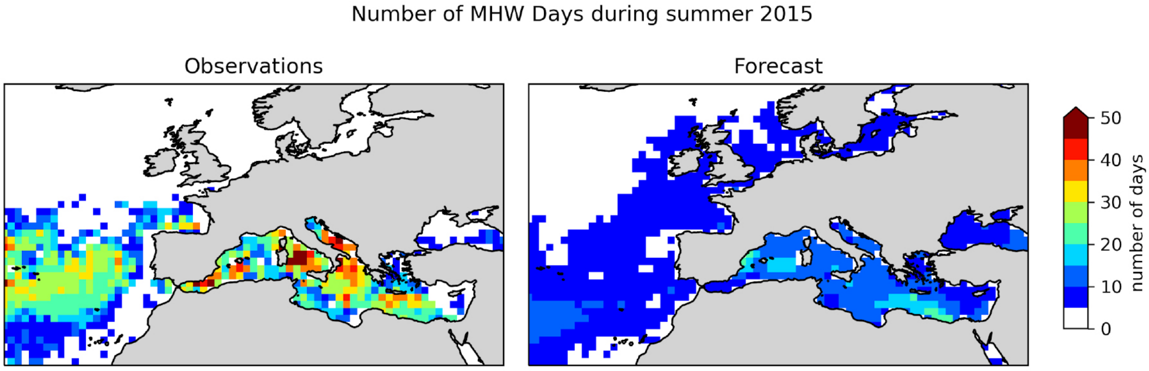

Figure 1: Number of days of marine heat waves between mid-May and September in 2015. Marine heat waves are defined as extreme anomalies in the sea surface temperature which last longer than 5 days. Left: satellite-derived observations from the European Space Agency Climate Change Initiative (ESA CCI) product. Right: forecast from the CMCC Seasonal Prediction System, made on 1st May 2015.

EARLY DEVELOPMENTAL EXPOSURE TO HEAT SHOCK IN RAINBOW TROUT (*Oncorhynchus mykiss*) DOES NOT AFFECT THE RESPONSE TO THE BACTERIAL PATHOGEN *Yersinia ruckeri* IN LATER LIFE BUT ALTERS THE RESPONSE TO PHYSICAL STRESSORS

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Introduction

Despite advancements in treatment and prevention, disease remains a major issue in aquaculture with implications for sustainability, welfare and food security. Genetic selection, often bolstered by genomic analyses, has proved successful at combating diseases in several cases, but manipulation of the epigenome in the context of disease prevention has seldom been explored. The propensity for vertebrate epigenomes to be manipulated by environmental stimuli, especially during critical windows of development, provides a potential opportunity for phenotypic selection by modifying epigenetic regulation of transcription. In a preliminary study, we have observed elevated heat shock protein transcriptional response to temperature stress in rainbow trout alevins previously exposed to heat shock during embryo development but not in naïve alevins. Here, we hypothesise that exposure to heat shock during critical windows of early embryo development may have the potential to alter disease susceptibility by modifying responsiveness to a pathogen via the manipulation of the epigenome.

Methods

To test this hypothesis, rainbow trout (*Oncorhynchus mykiss*) embryos were exposed to a 1 hour temperature shock during early development and then maintained under control conditions for the remainder of the study. Non-shocked (naïve) embryos were kept in parallel. At 106 days post hatch, these fish were infected with bacterial pathogen *Yersinia ruckeri*, the causative agent of enteric redmouth disease (ERM), via bath exposure. Control fish underwent a mock bath exposure to phosphate buffered saline (PBS) in which the bacteria were suspended for the disease exposure. Survival was monitored for 16 days, and liver samples were taken at 0, 19 and 43 hours post infection (hpi) for transcriptomic analysis.

Results

Yersinia ruckeri exposure led to rapid and substantial mortality in all infected groups, reaching 80% after 16 days in both heat shocked and naïve fish. Comparisons between heat shocked and naïve fish revealed that developmental exposure to heat shock had no significant effect on survival of fish exposed to *Y.ruckeri*.

Infection resulted in large changes in transcription with 4630 and 4145 differentially expressed genes identified at 19hpi and 43hpi, respectively (fold change > 2, $P < 0.01$), but few differences were identified between the responses of heat shocked and naïve fish. Interestingly, despite the apparent lack of influence of the heat shock pre-exposure on the response to disease, a differential response to the handling stress associated with the infection procedures was detected, suggesting that pre-exposure to heat shock has a modulatory effect on the response to subsequent physical stressors.

Conclusions

The data suggest that the developmental treatment applied here does not cause long term alterations relevant to disease susceptibility. However, different responses to handling stress during the disease challenge suggest that epigenetic manipulation may have occurred and may be affecting other stress response pathways.

EXPOSURE TO TEMPERATURE STRESS DURING EARLY EMBRYO DEVELOPMENT RESULTS IN GREATER HEAT SHOCK PROTEIN 70 INDUCTION IN RAINBOW TROUT (*Oncorhynchus mykiss*) LARVAE UPON RE-EXPOSURE

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Introduction

Aquaculture species are exposed to a wide array of stressors that limit production by impacting growth and survival. Despite genetic selection for traits improving growth or disease resistance, little attention has been afforded to selecting for robustness to the common stressors encountered during production. The propensity for the epigenome to be manipulated by environmental stimuli provides another potential platform for improvement of phenotypic traits in cultured species. However, whether intentional exposure to a stimulus can induce epigenetic alterations that result in beneficial changes in transcription and phenotype is unknown.

The present study aims to investigate the potential to intentionally induce epigenetic modifications and explore whether they result in altered responses to future stressors in an important aquaculture model species, the rainbow trout (*Oncorhynchus mykiss*). We hypothesise that exposure of rainbow trout embryos to temperature stress will result in significant alterations in transcriptional responsiveness upon re-exposure to temperature stress in later life, due to alterations in epigenetic patterns.

Methods

Our hypothesis was tested by exposing rainbow trout embryos to temperature stress during different developmental windows in the first week of development, while a control group was maintained in parallel. Post hatch, half of each group were either re-exposed to temperature stress for 24 hours or maintained in control conditions, and then sampled immediately. Real-time qPCR analysis of heat shock protein gene transcription (*hsp70a* and *hsp70b*) was used as a biomarker for responsiveness to temperature stress upon re-exposure.

Results and Discussion

Our findings revealed a greater responsiveness to temperature stress in fish that had been pre-exposed during embryo development. Furthermore, the developmental pre-exposure periods resulting in greater effects coincided with those overlapping with the putative epigenetic reprogramming period for fish species. These results suggest that it is possible to induce a greater ability to respond to temperature stress in rainbow trout by pre-exposing them during sensitive periods of embryo development. We are now investigating the epigenetic mechanisms accounting for these effects using reduced representation bisulfite sequencing. Our data highlights the potential for lasting memory of previous exposures to occur and for this to be used to induce altered phenotypic characteristics in cultured animals.

MACROALGAE AS A SOURCE OF SUSTAINABLE BIOPRESERVATIVES FOR FOOD SAFETY

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1. Introduction

With over 10,000 species identified to date, macroalgae experience incredible diversity; minerals can represent 7-36% biomass, lipids 1-5%, polysaccharides 15-76%, protein 5-47% (Cabral, Oliveira, et al., 2021). Macroalgae are exposed to extreme conditions, i.e. large variations in nutrients, temperature, pH, oxygen, and pathogens, leading to the production of secondary metabolites to increase their survival rate (Silva, Silva, Lourenco-Lopes, et al., 2020). These compounds have attracted the interest of the research community as they possess a wide variety of health properties (antioxidant, antimicrobial, anti-inflammatory, anticancer, anticoagulant amongst others), relevant for multiple biotechnological applications (Figure 1). Polysaccharides from macroalgae such as fucoidan, ulvan, galactan, and laminarin have been associated with antibacterial effects, most likely due to interactions with the bacterial cell wall, resulting in membrane leakage (Liu et al., 2017). These antimicrobial properties of macroalgae could play a valuable role as biopreservatives for multiple applications, including food (Cabral, Oliveira, et al., 2021).

With the rise in awareness of antimicrobial resistance in recent years, significant research has focused on alternative sources of antimicrobial compounds such as natural products (Quinto et al., 2019). Concurrent with the rise in focus towards alternative antimicrobials is the significant change in consumer food preferences, associated with concerns that chemically synthesised food preservatives pose dangers for human health, as well as concerns about the sustainability of their production. This has led to an increased interest by the research community in biopreservatives or natural compounds with antimicrobial activity which can be used as effective preservatives to improve food safety (Silva, Silva, Lourenco-Lopes, et al., 2020). This study aims to assess the antimicrobial activity of extracts of the macroalgae *Alaria esculenta* against microorganisms relevant for food safety.

2. Aim

The main aim of this study is to evaluate the antimicrobial activity against foodborne microorganisms of extracts of the macroalgae *Alaria esculenta*.

3. Methodology

Alaria esculenta was extracted using either ultrasound (US), microwave (MW), or ultrasound-microwave-assisted (UM) methods. Total phenolic content (TPC) and total soluble sugars (TSS) were determined via Folin-Ciocalteu reagent methods and phenol-sulfuric acid methods, respectively. Antimicrobial analyses included determining minimum inhibitory concentrations (MIC) and growth curves against microorganisms relevant for food safety and gentamycin as control following broth microdilution methods.

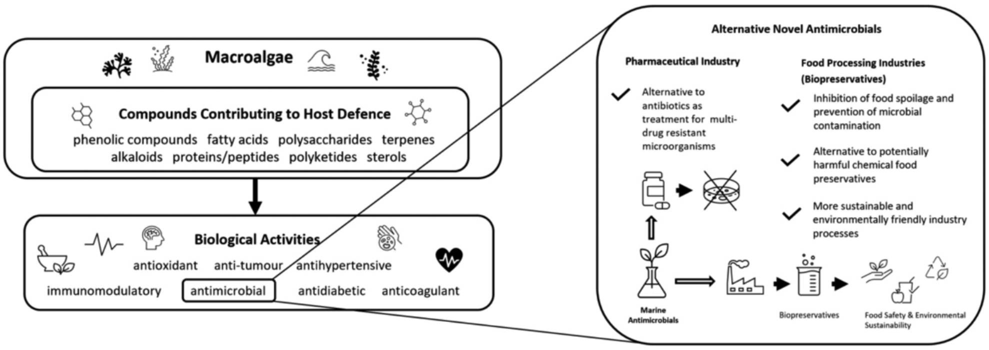

Figure 1. Schematic summarising secondary metabolites produced by macroalgae, their biological activities, and applications in pharmaceutical and food processing industries.

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4. Results and Discussion

Overall, the extracts evaluated contained low and similar levels of TPC (ranging from 1.27 to 1.64 mg gallic acid equivalents (GAE)/100 mg dried extract (DE) macroalgae) and protein (ranging from 4.64 to 9.93 mg/100 mg DE). The main differences in composition were appreciated in their contents of TSS, being 18.84, 19.14 and 32.68 mg glucose equivalents (GE)/100 mg DE. Antimicrobial activity was observed against *E.coli,* with MIC of 6.25 mg/ml in extracts prepared by US (TSS=32.68 mg GE/100 mg DE; TPC=1.27 mg GAE/100 mg DE), while the worst performing extracts with MIC of 12.5 mg/ml were achieved by those extracts generated by MW (TSS=18.84 mg GE/100 mg DE; TPC=1.5 mg GAE/100 mg DE).

5. Conclusions

This study indicates the potential of macroalgal polysaccharides generated using green extraction technologies as alternative antimicrobial agents against *E. coli*. Future research should focus on incorporating extracts in real food matrices to assess biopreservative potential.

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WWF’S GLOBAL AQUACULTURE STRATEGY

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Resource scarcity is upon us, and as we emerge from the global COVID-19 pandemic we are recognizing the razor’s edge we walk between supply chain function and collapse. Our contingencies for a sustainable food system are sparse, and the impacts of climate change are being observed, not theorized. But the importance of aquaculture continues to grow as a protein production system and as a mechanism for income generation. Aquaculture’s role in off-setting fishing pressures and creating a lower footprint protein are being questioned. Yet there remains great optimism that aquaculture can meet the expectations of creating a step-change in net food availability, beyond that of other proteins.

Considering the role of aquaculture in the future of food and importance of getting this right, WWF has embarked on a global network strategy to mitigate the negative impacts of aquaculture and amplify the beneficial factors. This first of its kind strategy seeks to increase the role of aquaculture as a livelihoods component, shift the research and development and demand from top-order predators to lower trophic species, halt forest and habitat conversion and increase the resource efficiency of production.

Discussion will focus on draft milestones and targets for aquaculture and the WWF Network approach to realizing the critical role of aquaculture in the broader food system.

EFFECT OF PERSONAL CARE PRODUCT (BUTHYLPARABEN, METHYLPARABEN AND PROPYLPARABEN) ON EMBRYONIC STAGES OF TENCH (*Tinca tinca*)

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Introduction

Personal care products (PCPs) are used daily by people around the world. Their entry into environment raises concerns about the potential threat to non-target organisms. Recently, there has been a growing interest in studying the properties of these pollutants. This is mainly due to their properties, which include, for example, the ability to bioaccumulate and also due to potential adverse effects on human health such as substances with mutagenic, carcinogenic and estrogenic activity (Brausch et al., 2010). Since these substances are constantly entering the aquatic environment, more attention must be paid to reveal their ecotoxicological potential. Moreover, it is known that fish are the most sensitive to aquatic contaminants between the period of hatching and onset eating exogenous food (Medkova et al., 2022). Therefore, it is necessary to know their toxicological properties on the early-life stages and their potential to affect health and welfare of aquatic organisms. The aim of this study was to determine the effect of PCPs (buthylparaben, methylparaben and propylparaben) on early life stages of tench (*Tinca tinca*), which is a fish commonly found in central Europe.

Materials and methods

The experiment was performed according to the Guideline for Test No. 236: Fish Embryo Acute Toxicity (FET) (OECD 2013) for the period of 96 hours with five different concentrations and control groups (ISO water and ISO water with solved ethanol). Tested concentration of methylparaben were 0.5, 50, 500, 5 000 and 100 000 µg/L, propylparaben and buthylparaben were tested at 0.1, 10,100, 1 000 and 100 000 μg/L. Fertilized eggs of tench (*T. tinca*) were selected using binocular microscope and distributed into 24 microwell plates. For each concentration plus control and control with solvent, 24 embryos were used. Tench (*T. tinca*) eggs were obtained from commercial farm Rybníkářství Pohořelice a.s. (Czech Republic). Tested substances were dissolved in water prepared according to ISO 7346 (ISO, 1996) with addition of ethanol (solvent). During the test the temperature was 24°C and the photoperiod was 12 hours light/12 hours dark. The solutions were changed every 24 hours and indicators of toxicity were recorded. We observed mortality, malformation rate, hatching and the heartbeat. At the end of the experiment, the samples of embryos were taken (8 replicates from each group) in order to study the effects of tested substances on selected genes expression using qPCR. For qPCR, only the lowest tested concentrations and their thousandfold were used. Statistical analysis was conducted using Unistat 5.6 for Excel (Czech Republic).

Results and discussion

Buthylparaben, propylparaben and methylparaben had a statistically significant negative effect on tench (*T. tinca*) mortality. Buthylparaben and propylparaben caused 100% mortality after 48 hours post fertilization (hpf) at the highest tested concentration. Also, at concentrations of 100 and 1000 µg/L the mortality reached 21% of embryos exposed to these concentrations of butylparaben. Similarly, Merola et al. (2020) described high mortality of zebrafish (*Danio rerio*) after 48h long exposure to buthylparaben. Concerning methylparaben, concentration of 100 000 μg/L caused mortality of 45% specimen. Moreover, concentration 5 000 μg/L caused 21% mortality and concentration 500 μg/L caused mortality of 25%

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embryos. On top of that a 96hours exposure to methylparaben revealed increased malformation rate. At the concentrations 5 000 and 100 000 µg/L of methylparaben malformation rate of 25% was observed. The most common malformations were underdeveloped head, heart oedemas, and deformations of tail and spine. Similarly, Dambal et al. (2017) observed malformations such as pericardial oedema, bent spine and blood cell accumulation on embryos of zebrafish (*D. rerio*) that were exposed to buthylparaben. Buthylparaben and propylparaben did not have any effects on the occurrence of malformations. None of the tested substances had any significant effect on the time of hatching. Embryos were hatched at all concentration and all tested substances at 72 hpf. Both butylparaben and propylparaben influenced the expression of the gene *hsp70*, whose expression is known to be markedly induced in response to environmental stresses. Also, methylparaben and propylparaben altered the exposure of *cyp1,* which is used as biomarker of aquatic pollution.

Conclusion

Based on the results of this study, buthylparaben, propylparaben and have at certain concentrations the ability to negatively affect development and welfare of non-target fish and other water organisms.

Acknowledgments

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MEALWORM (*Tenebrio molitor*) IN DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*): A STRATEGY TO FACE THE OMEGA-3 PROBLEM OF INSECT-FED FISH

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Introduction

The use of insects shows an interesting potential as an alternative to fishmeal in aquafeeds, but tends to diminish the omega-3 (ω-3) fatty acid content in fish fillets (De Haro *et al.*, 2015; Fabrikov *et al.*, 2021; Jeong *et al.*, 2022). Some experiences have attempted to counter this drawback through different strategies (Sealey *et al.*, 2011; Jeong *et al.*, 2021). The present work tested one of them by using an ω-3 enriched oil at different dietary inclusion levels in rainbow trout, and also analysed if that strategy could have other side effects on fish physiology.

Material and Methods

Four isoproteic (45.5%) and isolipidic (17%) diets were tested: one control (C) based on fishmeal as main source of protein, and three with a 50% replacement of fishmeal (18% feed inclusion) with *Tenebrio molitor* meal and an increasing level (0%, T50; 3.1%, TV1; and 7.2%, TV2) of an experimental algal oil rich in ω-3 (EPA and DHA) that partially replaced fish oil. 400 rainbow trout specimens (*Oncorhynchus mykiss*; 4 tanks per diet, 25 fish per tank) with an initial body weight of ~46 g were fed the experimental diets in a recirculating aquaculture system in optimal and controlled conditions during 89 days, until they reached a final body weight of ~415 g. At the end of the growth trial, fillet fatty acids profile, liver redox status, and liver histomorphology were analysed.

Table 1. Effects of experimental diets on fillet and liver of rainbow trout

Fatty acid profile (%/total)	**C**	**T50**	**TV1**	**TV2**	**SEM**
Eicosapentaenoic acid (EPA)	3.29^{c}	2.34^{d}	4.09^{b}	5.33^{a}	0.17
Docosahexaenoic acid (DHA)	18.95^{a}	15.22^{b}	17.51^{ab}	19.94^{a}	0.74
Total omega-3 (ω-3)	24.95^{ab}	19.65^{c}	24.71^{b}	28.65^{a}	0.92
Liver redox status	**C**	**T50**	**TV1**	**TV2**	**SEM**
Superoxide dismutase (U/mg protein)	282.36	271.14	299.62	277.21	10.5
Catalase (U/mg protein)	325.45	342.72	378.66	368.94	20.26
Glutathione peroxidase (mU/mg protein)	16.53^{a}	13.1^{b}	10.41^{b}	10.26^{b}	0.68
Glutathione reductase (mU/mg protein)	7.73	6.3	6.29	6.65	0.52
Glucose-6-phosphate dehydrogenase (mU/mg protein)	57.74	50.21	44.28	44.08	7.37
Malondialdehyde (nmol/g tissue)	65.06^{b}	65.06^{b}	99.54^{a}	111.32^{a}	7.40
Liver histomorphology	**C**	**T50**	**TV1**	**TV2**	**SEM**
Hepatocyte nuclei width (µm)	2.05	2.04	2.09	2.1	0.04
Hepatocyte cytoplasm width (µm)	4.45	4.42	4.59	4.62	0.05
Lipidic accumulation in hepatocytes	+	+	++	++	-

C: Control diet (no fishmeal replacement); T50: 50% fishmeal replacement with *Tenebrio molitor*; TV1 and TV2: 50% fishmeal replacement with *Tenebrio molitor*, and supplemented with 3.1 and 7.2% (respectively) of experimental algal oil. $^{a, b, c, d}$ indicate significant differences ($p<0.05$) between diets. Data are expressed as mean ± standard error of the mean (SEM).

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Results and discussion

Growth performance (not included) did not show significant differences among experimental groups, which followed the tendency of a previous study in this species (Melenchón *et al*., 2022). Fillets of fish fed with T50 showed an overall decrease in ω-3 fatty acid content, while TV1 and TV2 had this decrease corrected or reversed, showing even higher levels than C diet in some cases. Interestingly, TV1 and TV2 showed increased lipid accumulation within hepatocytes, which has already been described in other insect-fed fish trials (Randazzo *et al*., 2021). Due to this lipid storage, a situation of oxidative stress occurred in the liver of TV1 and TV2, represented by an increase in malondialdehyde levels linked to a decrease in glutathione peroxidase activity. Perhaps, additional supplementation of these diets with antioxidants would have been necessary.

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CHANGES IN INTESTINAL MICROBIOTA AFTER THE INCLUSION OF WHOLE AND PARTIALLY DEFATTED YELLOW MEALWORM (*Tenebrio molitor*) IN DIETS FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The use of insects shows an interesting potential as an alternative to fishmeal in aquafeeds. Since the intestine is the main organ involved in the absorption of nutrients, gut health plays an important role in fish growth. Also, the study of intestinal microbiota shows potential on this matter, because different microorganisms can influence the processing of nutrients (Semova *et al*., 2012; Wu *et al*., 2012). The present work evaluated the microbiota of rainbow trout distal intestine after a feeding period with one whole insect meal, and a partially defatted version of the same insect, yellow mealworm (*Tenebrio molitor*).

Material and Methods

Three isoproteic (45.5%) and isolipidic (17%) diets were tested: one control (C diet) based on fishmeal as a main source of protein, and two with a 50% replacement of fishmeal (18% feed inclusion) with *Tenebrio molitor* meal, one whole (T diet), and one partially defatted (TD diet). 300 rainbow trout specimens (*Oncorhynchus mykiss*; 4 tanks per diet, 25 fish per tank) with an initial body weight of ~46 g were fed the experimental diets in a recirculating aquaculture system in optimal and controlled conditions during 89 days, until they reached a final body weight of ~415 g. At the end of the growth trial, samples of intestinal digesta were taken for microbiota analyses.

Table 1. Effects of experimental diets on the microbiota of intestinal digesta samples of rainbow trout

Microbiota (% OTU/total)		**Experimental diets**			
Family level	**Genus level**	**C**	**T**	**TD**	**SEM**
Bacteroidaceae	*Bacteroides*	3.7	3.61	2.59	0.48
Porphyromonadaceae	*Falsiporphyromonas*	7.47	6.46	5.11	0.89
Nostocaceae	*Nostoc*	13.18^a	11.91^a	7.56^b	0.6
Bacillaceae	*Bacillus*	0.21^b	2.94^a	0.25^b	0.14
Paenibacillaceae	*Brevibacillus*	0.02^b	8.06^a	0.004^b	0.49
Enterococcaceae	*Enterococcus*	0.23^b	1.78^a	0.27^b	0.18
Streptococcaceae	*Streptococcus*	7.45	7.16	8.75	0.46
Peptostreptococcaceae	*Helcococcus*	1.18^a	0.86^b	1.39^a	0.06
	Peptostreptococcus	20.23^b	15.84^b	27.04^a	1.69
Peptoniphilaceae	*Peptoniphilus*	14.86^b	13.06^b	18.47^a	0.8
	Citroniella	2.01^b	1.55^c	2.27^a	0.06
	Other	29.46	26.77	26.3	-

OTU: Operational taxonomic unit; C: Control diet (no fishmeal replacement); T: 50% fishmeal replacement with whole *Tenebrio molitor*; TD: 50% fishmeal replacement with partially defatted *Tenebrio molitor*. $^{a, b, c}$ Indicate significant differences ($p<0.05$) between diets. Data are expressed as mean ± standard error of the mean (SEM).

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Results and discussion

Growth performance (not included) did not show significant differences among experimental groups, which followed the tendency of a previous study in this species when fed yellow mealworm (Melenchón *et al*., 2022). Several changes in microbiota composition are described in Table 1. Among them, a significant increase in *Bacillus sp*., *Brevibacillus sp*. and *Enterococcus sp*. was highlighted for T diet. Lactic-acid bacteria (such as *Enterococcus*) and bacteria from the *Bacillus* genera are known for having probiotic properties in fish (Chang and Liu, 2002; Capkin and Altinok, 2009), which is positive from an applicative point of view. Similar results have been found in other experiences with insect-fed fish (Józefiak *et al*., 2019; Gaudioso *et al*., 2021), but since our experiment described this increase only for T diet, it is possible that insect fat is more relevant for the growth of these bacteria than other insect components like chitin. In this vein, there was also an increase in amino acid fermentative bacteria (*Peptostreptococcaceae* and *Peptoniphilaceae*) and a decrease in *Nostoc sp*. in TD diet, showing the relevance of the nature of the fat in the microbiota profile.

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THE USE OF DIET ENCAPSULATION AS A VALUABLE TOOL TO IDENTIFY THE POTENTIAL OF NOVEL FISH DIETS

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Introduction

Fish can choose their diets and regulate feed intake through “nutritional wisdom”, which is controlled by behaviour, learning and physiological processes, such as the evaluation of the palatability, oral sensation and sensory properties of feed. In aquaculture research, normally, the effects of new diets are performed using growth trials, mainly based on physiological mechanisms. Although this methodology is efficient, it does not consider fish preferences. Moreover, the gustative and olfactory properties of traditional extruded or pelleted diets (texture, taste, odour and flavour) can have functional effects to inhibit or stimulate feed intake. Therefore, to provide a more complete perspective on the potential of novel diets, growth trials may be complemented with other methods.

Self-selection methods have the potential to become valuable tools for the design and ingredient selection of new diets. These methods allow fish to choose which feed most suits their nutritional needs. Diet encapsulation has been used as a promising approach focused only on the nutritional content of feeds, as it packages diets inside eatable gelatine capsules, with the same orosensory characteristics, which masks the organoleptic properties of feeds. Thus, encapsulation allows fish to express their natural behaviour, without the effect of chemosensitivity.

The main objective of this work was to understand the behaviour response and feed preference of Nile tilapia (*Oreochromis niloticus*), using the self-selection method of diet encapsulation, towards the acceptability of innovative sustainable feed ingredients, spirulina (*Arthrospira platensis*) and quinoa (*Chenopodium quinoa*).

Methods

Three experimental diets were formulated and produced at the University of Murcia with distinct proximal compositions. Diet A had casein and dextrin (34% and 30%, respectively) as main ingredients and served as a proxy for a purified diet. Diet B was mainly composed of spirulina (58%), while Diet C had different doses of spirulina and quinoa (46% and 20%, respectively). Diets were given as multi-coloured capsules to 7 individual fish (*O. niloticus*) of 331.88 g ± 31.39 g (mean ± S.D.) for 35 days. To determine daily intake, the total number of uneaten capsules corresponding to each diet was counted for every fish and subtracted from the total number of capsules given. After a determined diet exhibited statistically significant differences (means compared using SPSS version 23.0 by one-way ANOVA, followed by a Tukey’s post-hoc) over the others, feeds were switched between capsule’ colours to reduce the possible preference for a specific colour.

Results

After 10 days of relatively similar diet preferences, fish progressively increased their intake and selection of capsules containing diet C, while simultaneously decreasing that of diets A and B (remained at an average level of 30% and 31%, respectively) (Fig. 1). When the preference for diet C reached values (maximum of 44%) that showed statistically significant differences ($p < 0.05$) for several days (day 11 – 16), the colour of the capsules associated with each diet rotated. Initially, this change decreased the ingestion of diet C, while conversely increasing diet A and B. Until day 29, the preference of fish towards the diets remained at a steady state, until a clear consistency and preference for diet C was once again achieved (between 40.4% and 47.2%; $p < 0.05$).

During days with statistically significant differences between feeds, diet C (1.63 capsules/100g BW; $p < 0.05$) was ingested around 46.8% and 44.2% more than diet A (1.13 capsules/100g BW) and B (1.11 capsules/100g BW), respectively (Fig. 2).

Conclusions

Diet C (with spirulina and quinoa) leads to a better feed intake than diet B (only with spirulina), while the purified diet A (with casein and dextrin) resulted in the lowest intake. Nile tilapia was able to select one of the given feeds based only on post-ingestion and absorption signals. Diet encapsulation trials allow fish to express their behaviour, thus these methods may be considered in the initial screening of potential new aquaculture feeds.

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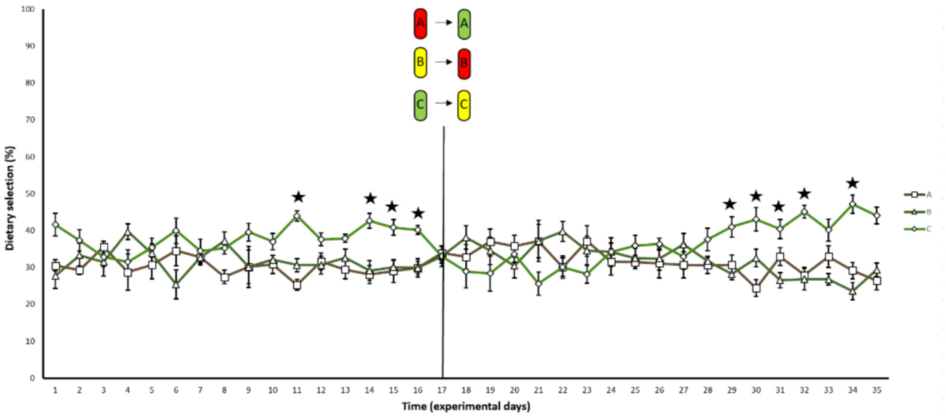

Figure 1 – Evolution of average daily intake of three diets (Diet A, B and C) by Nile tilapia, expressed in percentage of the total number of capsules ingested for 35 days. The content of the capsules changed on day 17. Values represent the mean ± S.E.M. of 7 individual fish. Stars represent significantly different values (ANOVA, $p < 0.05$).

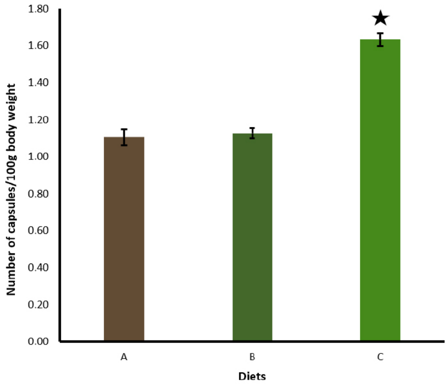

Figure 2 – Average daily intake of three diets (Diet A, B and C) by Nile tilapia, represented by the number of capsules ingested per 100g of body weight during days with statistically significant differences between diets. Values represent the mean ± S.E.M. of 7 individual fish. The star represents significantly different values (ANOVA, $p < 0.05$).

Acknowledgements

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UNDERSTANDING THE VALUE OF FRESHWATER AQUACULTURE AND FISH PROCESSING BYPRODUCTS THROUGH AGROINNOVATIVE APPROACH & TECHNOLOGICAL SOLUTIONS

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Introduction

The SEA2LAND EU project (2021-2024) aims to provide solutions to help overcome challenges related to food production, climate change and waste reuse. This project will meet this challenge by improving and adapting technologies for nutrient recovery to produce bio-based fertilisers (BBFs) and Tailor Made fertilisers (TMFs) from fishery and aquaculture by-products generated in Europe. Therefore, SEA2LAND will contribute to independence and secure the supply of nutrients to European agricultural systems and will promote the production of large-scale fertilizers (from non-imported raw materials), based on the circular economy model to transform by-products into nutrients for crops. Particularly, CAVIAR PIRINEA together with some research partners are mading available extended catalogue of sidestreams charaterization, which are routinely sold to waste managing companies with no valorisation income derived. The technological approaches developed herein by BETA are revealing that within a private aquaculture production scenario of 3.100T fish/year (i.e., rainbow trout (*O. mykiss*) and sturgeon (*Arcipenser sp.))* and 1.902T of fresh processed products/year (i.e., fish, roe and caviar) => 69m^3 fish sludge, 96T fish víscera and 200T of non-conforming fish/year could respectively be valuated through some of the current research techniques in progress. Likewise, the project could show that every increased productivity as derived from intensification in aquaculture and/or fish processing practices will not only lead to a subsequent huge, but also economically recoverable production of agro innovative byproducts. The solutions developed are expected to reduce the soil nutrient imbalance in Europe.

Material and Methods

An innovative technological approach is being evaluated within WP4 for the valorization of aquaculture by-products (fish sludge and dead fishes). The **SLUDGE** is separated into solid and liquid fractions. Solid fraction valorization is conducted by applying biodrying processes, aiming to reduce the water content and obtain a nutrient-rich biodried product to be used as an organic amendment. The combustion of this product to recover phosphorus from the ashes to produce **phosphoric acid** is also expected. The feasibility of recovering the volatilized ammonia during the biodrying to produce **ammonium salt** is also under current evaluation within the project. The liquid fraction from sludge is being valorized by using several concentration processes. The method consists of an ultrafiltration (UF) system to separate solid particles present in the liquid fraction. The permeate produced by UF passes through the reverse osmosis (RO) system to concentrate the nutrients. The RO concentrate generated is subsequently processed in a Freeze Concentration (FC) unit, where the water is crystallized, and the **nutrients** are recovered in a nutrient-rich concentrate product. Moreover, a high-quality RO permeate with potential to be reused in the aquaculture industry facilities is also produced.

Results

Sludge produced from freshwater aquaculture systems can be successfully valorized by a portfolio of technologies to recover nutrients on a dry basis, as follows for example: (i.) Solid fraction of freshwater sludge: 7.0% TKN, 0.4% TP, and 0.1% K. and (ii.) Liquid fraction of freshwater sludge: 0.33 g N/L, 0.02 g TP/L, and 0.02 g K/L. Dead fish is being valorized as a co-substrate during the treatment of the solid fraction from freshwater aquaculture sludge. The specific use of trout as a co-substrate is showing to be very potential because it presents high organic matter content and valuable and recoverable nutrients as N (4.4% TKN) and P (0.4% TP) on a dry basis.

Likewise, 26 private and public stakeholders involved in 8 technical work-packages and with expertise in the fields of agriculture, aquaculture, fisheries and fish processing from all over Europe, will continue developing through the end of 2024, comprehensive data sets relating to (i.) agriculture and fertilization; (ii.) side-streams characterization and technology development; (iii.) food waste and circular economy; (iv.) environmental performances and constraints; (v.) sustainability assessment and business models.

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Conclusions

The physicochemical characterizations herein are making evident the **feasibility of recovering nutrients from dead fish and sludge produced in aquaculture companies** and it could represent a promising option for the production of bio-based fertilizers (BBF) from aquaculture by-products (further details on freshwater vs. marine, in progress).

Furthermore, the potential application of **these BBF could reduce the costs associated with sludge management**, bringing both cost reduction a new market opportunity, for the aquaculture sector.

CAVIAR PIRINEA and BETA Research Centre (University of Vic, Catalonia) will continue working on **nutrients recovering and pilot valorisation activities**, for both aquaculture and trout processing activities.

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Acknowledgments

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CARBON BIOAVAILABILITY AND NITRATE DETERMINE H_2S PRODUCTION POTENTIAL IN SLUDGE COLLECTED FROM ATLANTIC SALMON IN RAS

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Introduction

Hydrogen sulphide (H_2S) has been suspected as a potential cause for sudden, unexplained mass mortalities in RAS producing Atlantic salmon postsmolts (Hjeltnes et al., 2019). Marine or brackish RAS are often rich in organic matter, creating ideal conditions for heterotrophic, sulphate-reducing bacteria. In the presence of nitrate and absence of O_2, organic matter should be preferentially used in denitrification, where nitrate is reduced to inert N_2 gas. In the absence of nitrate and oxygen, biodegradable organic matter can be respired with sulphate from sea water, creating highly toxic H_2S. Therefore, maintaining high concentrations of nitrate in RAS is commonly considered as mitigation strategy to reduce the risk for H_2S-events (Højgaard, 2017). However, it is currently not clear how much nitrate is required to prevent the formation of H_2S in sludge. In this study, we have investigated the total potential of H_2S production of sludge collected in RAS with Atlantic salmon in continuously stirred batch reactors. The aim was to investigate the stoichiometric relationship of biodegradable organic matter and nitrate on reaction rates and the total potential for H_2S-generation in RAS.

Material & methods

Sludge was collected as a compound sample from swirl separators connected to the bottom outlet of three RAS tanks from the same system, stocked with Atlantic salmon post smolts of approximately 280 g and operated with brackish water (12 ppt). The sludge was then homogenized and divided between respirometric cBOD assays (41 days, 20°C) and continuously stirred batch reactors to investigate H_2S production of sludge in presence of nitrate. After deoxygenating brackish water (12 ppt) with N_2 gas, 12 batch reactors were prepared with a fixed sludge dose (1% V/V sludge, 0.93 g DM/L, 1.17 g COD/L), and adjusted to variable nitrate concentrations (0, 30, 60 ,120 mg NO_3N/L) in triplicate . The airtight batch reactors (V = ~2 L) were then incubated at 12.4 °C ± 0.2 over 90 days. H_2S production was measured with the "Sulfulla" wet-chemical method previously published by Letelier-Gordo et al. (2020).

Results & discussion

Respirometric assays indicated that about 82% ± 1.7% (n = 4) of the COD in sludge was biodegradable, resulting in cBOD/N ratios of 30.9, 15.5 and 7.8 g cBOD/g N in our treatments with initial nitrate concentrations of 31, 62 and 123 mg NO_3N/L, respectively (COD/N 37.7, 18.9, 9.5). We found that nitrate did not only delay the initial H_2S response in our reactors (5 days for 0 and 30 mg/L NO_3N vs. 7 and 9 days for 60 and 120 mg/L NO_3N, respectively), but also reduced the overall amount of S^{2-} at the end of the experiment (38 g S^{2-}/L sludge and 24.4 g S^{2-}/L sludge for 0 and 120 mg NO_3N/L, respectively). Nitrate depletion in the treatments was confirmed after 3 days for 30 and 60 mg NO_3N/L and 5 days for the 120 mg NO_3N/L treatment, initial S^{2-} production rates were relatively similar between treatments (~0.6 g S^{2-}/L sludge/d, or 6.2 g S^{2-}/kg cBOD/d).

Taking the cBOD/COD ratio of 0.82 into account, we can account for close to 100% of the organic matter supplied to our reactors. Using a stoichiometric ratio of 2.86 g cBOD/g NO_3N for denitrification (Metcalf & Eddy, 2004), we calculated that about 30% of the biodegradable COD was used for denitrification in the 120 mg NO_3N/L treatments after 60-90 days (Fig. 1). Consequently, 2.67 ± 0.161 g of biodegradable COD was used to generate 1 g S^{2-} in our reactors, which closely matches the theoretical stoichiometric ratio of 2.67 g COD/ g S^{2-} for sulphate reduction of Tchobanoglous et al. (2004).

Conclusions

The presented results confirm that nitrate does delay and reduce H_2S production in continuously mixed batch reactors, showing a delay of 2-5 days in H_2S response of sludge collected from Atlantic salmon in RAS after the depletion of an alternative electron acceptors (O_2, NO_3) to sulphate at 12 °C. Furthermore, we highlighted the importance of carbon bioavailability in sludge for assessing H_2S risks in RAS by confirming the basic stoichiometric assumptions underlying organic matter degradation. Although nitrate can delay and reduce the H_2S response in mixed systems, more research is required to understand H_2S production dynamics in sediments and biofilms typical for RAS where diffusion could be a rate-limiting factor.

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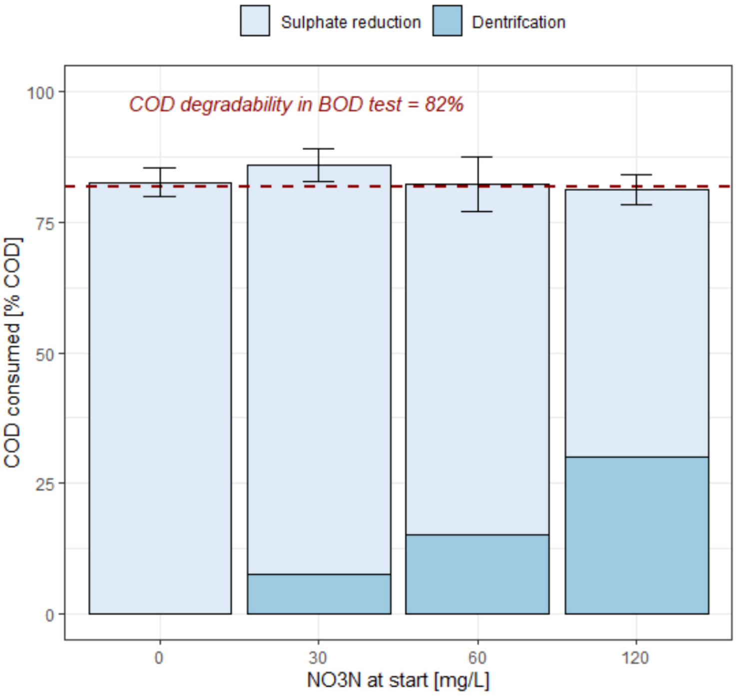

Figure 1: Allocation of biodegradable COD for the reduction of nitrate and sulphate in ideally-mixed batch reactors with sludge from Atlantic salmon (12 °C). The red line indicates COD degradability, determined in respirometric cBOD assays (cBOD, t = 41 d).

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VACCINATION OF NILE TILAPIA WITH INACTIVATED IMMERSION AUTOGENOUS VACCINES REDUCE MORTALITY AND CLINICAL SIGNS PRODUCED BY AN EXPERIMENTAL INFECTION BY INTRAGASTRIC ROUTE WITH *Francisella noatunensis* SUBSP. *Orientalis*

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Introduction

Francisellosis induced by *Francisella noatunensis* subsp. *orientalis* (FNO), is a major threat to the global tilapia industry. There are no licensed vaccines currently available against the disease in tilapia. This study pretended to evaluate the efficacy of a newly developed inactivated autogenous vaccine against francisellosis.

Materials and methods

Nile tilapia fingerlings (1.7 g) free of FNO were recruited for the study. Group A (n=65) and B (n=65) were vaccinated by immersion with autogenous whole-cell inactivated and adjuvanted mono and tri valent FNO vaccines; these were prepared with FNO strains respectively homologous and heterologous to the challenge strain. Group C (n=43) and D (n=22) were vaccinated by immersion with a sterile physiological solution. Group A-D were infected with FNO ($3x10^7$ CFU) by intragastric route 25 days after vaccination and previous anaesthesia. During the 21 days following the infection (dpi) the animals were maintained at 22ºC and the health outcome was monitored twice a day. The number of clinical signs observed in each tank per 100 fishes throughout the study was used to calculate the Area Under the Curve. The efficacy of the vaccine was calculated as Relative Percentage Survival (RPS).

Results

The infection of group C produced mortality (65.12%) and clinical sign commonly observed in francisellosis. Results showed groups A-B showed reduced the mortality and thus achieved RPS of 71.65% and 55.11%. Moreover, the AUC of clinical signs was proportionally reduced compared to group C about 77.49% and 83.92%.

Conclusions

Previous authors demonstrated that immersion whole-cell inactivated vaccines can achieve about 50% of RPS when administered in fingerings and challenged with FNO by intraperitoneal route [1].

The gastrointestinal route is a natural getaway for bacterial infection. This study demonstrate it can be used to test the vaccine efficacy. The tested immersion vaccines reduced the mortality as well as the clinical signs produced by the FNO infection in fingerlings and achieved RPS which can be higher than 50%.

Therefore, the tested vaccine demonstrated to be a promising solution to control francisiellois in nile tilapia.

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EFFECT OF A NUTRIENT ABSORPTION ENHANCER AND BILE ACIDS IN ENERGY REDUCED DIETS FOR ATLANTIC SALMON PRE-SMOLTS

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Introduction

Salmonids are very efficient in using lipids and proteins as energy source[1]. Dietary protein in excess to what is needed for growth and maintenance, is used as an energy source[2]. Protein is the more expensive part of a salmon diet while lipids are a comparatively cheaper ingredient. Replacing the energy resulting from an excess of protein in the diet by energy generated from lipids, while maintaining the same growth is known as the protein sparing effect of lipids[3]. However high levels of vegetable oil inclusion have shown to increase lipid accumulation in the liver[4] and cause issues such as altered immune response and disease resistance. In addition, the cost of vegetable oil has increased during last few years and thereby high energy diets increase the feed cost. Reducing the energy content of feed can improve profitability of a salmon farm and can help to overcome liver health issues. However, this would impact the overall growth performance. In this study, a nutrient absorption enhancer was used in low energy diets to enhance the performance and liver health in Atlantic salmon (*Salmo salar*) pre-smolt.

Materials and methods

The positive control diet was formulated to contain 49% protein, 26% lipids and a gross energy of 24 MJ/Kg. The negative control diet the was formulated to contain 49% protein, 20.5% lipids and a gross energy of 23 MJ/Kg. For the test diets, negative control diet was supplemented with 1kg/ton of an additive containing lysophospholipids and butyrate (Aquatria^{TM}) or with 1kg/ton bile acid. The study was conducted at the Experimental Aquaculture Unit, Catholic University of Temuco. Atlantic salmon pre-smolt of approximately 20g were stocked in 100-liter tanks, 34 fish per tank, 3 tanks per treatment. The fish were fed 3% of the biomass twice per day during the test period of 81 days. At the end of the study, the fishes were weighed to evaluate growth performance, and calculate weight gain, specific growth rate (SGR) and protein efficiency ratio (PER). Feed intake was recorded on a daily basis and feed conversion ratio (FCR) was calculated.

Livers of 3 fish of each tank were processed for histopathological evaluation of the degree of lipidosis. The classification described by Bernet *et al*.[4] and modified by Rosenthal *et al*.[5] was used, which defines five degrees of hepatic lipidosis ranging from grade 0 to grade 4.

Results

Growth results are summarized in Table 1.

Discussion and conclusion

Reducing the lipid and energy content of the positive control diet resulted in a decrease in specific growth rate and protein efficiency ratio and an increase in feed conversion ratio. Supplementation of the negative control diet with 1000 ppm of Aquatria reverted the negative impact of the reformulation. These results demonstrate that this additive offers the possibility to reduce the level of vegetable oil in a salmon diet without affecting the growth.

In addition, the improvement in liver condition that is observed with the fish fed the reduced energy diet, was further augmented by the addition of this additive.

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Table 1. Overview of growth results. Results are the average of 3 tanks ± standard deviation

	Pos Control	Neg control	lysolecithin and butyrate	bile acids
weight gain (g)	22.8 ± 2.2	21.4 ± 0.7	23.0 ± 0.5	21.2 ± 4.1
final weight (g)	42.6 ± 2.1	41.3 ± 0.7	43.1 ± 0.6	40.6 ± 4.0
SGR (%/day)	0.95 ± 0.07	0.90 ± 0.02	0.95 ± 0.02	0.91 ± 0.13
FCR	1.55 ± 0.14	1.63 ± 0.07	1.51 ± 0.04	1.72 ± 0.42
PER	1.32 ± 0.12	1.25 ± 0.06	1.34 ± 0.03	1.22 ± 0.26

Overview of the scoring of each fish can be found in Table 2, with grade 0 showing no vacuoles with defined border in the cytoplasm and grade 4 showing severe presence of macro and micro vacuoles in the cytoplasm, flattened nucleus displaced to the periphery and loss of structure.

Table 2. Grading of liver lipidosis seen with fish fed the different experimental diets, with grade 0 being no lipidosis and grade 4 severe lipidosis.

Grade	pos control	neg control	lysophospholipids and butyrate	Bile acids
0			3	
1		5	6	5
2	2	4		4
3	2			
4	5			

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INVESTIGATING THE USE OF DEFATTED *Desmodesmus* IN ATLANTIC SALMON FEEDS DURING THE GROW-OUT PHASE IN SEA

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Introduction

The salmon farming industry requires sustainably produced feed ingredients of good nutritional value. Microalgae are excellent candidates for salmon feed ingredients as the algae industry is finding ways to commercially produce them in huge volumes and their nutritional profile is expected to favour the growth and welfare of Atlantic salmon. The major drawback linked to microalgae is the high production cost and hence, the produced biomass should also be diverted to manufacture high-priced pharmaceutical and cosmetic products for human use. Thus, effective utilization of the biomass and improvements in the production methods will progressively reduce the price of algae to a point where the cost will not be a deterrent for their utilisation in animal feeds. It is important that every microalgal fraction left as waste from the production of well-established, commercial products is used up to contribute to a circular economy.

In the present study, we explored the inclusion of defatted *Desmodesmus* in salmon feeds, a biorefinery co-product which is rich in proteins, carbohydrates, minerals and vitamins. In the experiment, performed at Langholmen, Norway (GIFAS), we investigated whether this ingredient has any effect on Altantic salmon key performance indicators (KPIs), flesh quality and composition, and fillet oxidative stability during cold storage.

Materials and methods

Six net pens (5 x 5 x 5 m) in sea were stocked with 80 salmon each (mean weight 1,8 kg), and the trial was run in triplicate pens. Two isoproteic and isolipidic feeds were fed to Atlantic salmon, *Salmo salar*: CTRL (control) and DX (feed containing 7.5% defatted *Desmodesmus* sp.). Daily feeding was carried out by hand and to satiation. Uneaten pellets were collected after every meal, using an underwater funnel and vacuum pump and quantified for accurate assessment of feed consumption.

At the trial start (November 2019), bulk weighing was performed to understand the total biomass in each pen. Each experimental fish was weighed when the trial was terminated in June 2020; i.e., once the size of the fish was more than double the initial size. The fat content and colour of the fillet was measured photometrically using PhotoFish (AkvaGroup AS, Norway). Intestinal morphology was evaluated by histology. Whole body was collected for assessing the proximate composition and energy content. Finally, the oxidative stability of the flesh was assessed during cold storage for the duration of 6 days (Dey, 2020).

Results

The feeding study was run for 197 days, with fish of initial weight 1,83 kg ± 0,02. The average weight of the fish at the end of the feeding period was 4,16 kg ± 0,17. The growth indicators SGR and TGC were not significantly different across dietary treatments. However, the FCR was significantly better in CTRL (1,13) than in DX (1,20). The K factor was also slightly but significantly higher in CTRL fish (1,32) compared to DX fish (1,26), indicative that CTRL fish had a more round (fat) shape.

Colour (in terms of both astaxanthin concentration and SalmoFan) and fat content in the fillet were not significantly different across the diet groups. Furthermore, whole body proximate composition did not show significant differences across dietary treatments.

Significant differences were noted in the fatty acid composition of the fillets from the two groups; the EPA level was lower in the DX group. However, the level of polyunsaturated fatty acids (PUFAs) was not significantly different between the 2 groups; including the DHA level.

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Finally, intestinal health was analysed by histological methods. The intestinal fold height and villus width were significantly higher in the DX group, indicating superior gut health. The width of the lamina propria was significantly lower suggesting the absence of inflammation.

The oxidative stability of fillet on cold storage indicated that although the primary oxidation product was higher in DX group on the 6th day, the secondary oxidation product did not show such a trend.

Conclusions

Results from this trial have revealed that defatted microalgae are suitable ingredients for salmon feeds. Differences in fatty acid composition across diets suggest that, even though the microalgal ingredient was defatted, it might still contain some lipids to justify these variations. Importantly, the microalgal diet DX gave superior gut health parameters than the CTRL feed. The observation on the fillet oxidative stability needs to be explored further.

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Acknowledgements

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THE OPTIMUM HARVEST TIME FOR CULTIVATED KELP SPECIES (*Saccharina latissima* AND *Alaria esculenta*) DEPENDS ON THEIR INTENDED APPLICATION

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Interest and demand in cultivating seaweeds has been growing rapidly in Western Europe, driven by developing expertise and markets for food and non-food (cosmetics, pharmaceuticals, biofuel, etc.) applications. Kelp species such as the sugar kelp *Saccharina latissima* are most suited and prioritized for commercial culture due to their high growth potential and content of valuable compounds. Thus, they are favoured by research efforts informing the industry, answering questions such as the best time for harvest whilst often using biomass yield alone as the decisive factor.

We cultivated two kelp species (*Saccharina latissima* and *Alaria esculenta*) at our experimental seaweed farm on the West coast of Scotland and performed biweekly consecutive harvests throughout the harvest season (early March to July 2020; $n = 9$ harvest timepoints). Seaweed appearance (blade length and –width, fouling index), biomass yield, nutritional profile (%DW proteins, lipids, etc.) and composition (trace minerals, heavy metals, iodine) were followed over time and in conjunction with prevailing environmental conditions (seawater temperature, light, nutrient, and chlorophyll-a concentrations).

Overall seaweed appearance, quantity, and quality differed across species and harvest times. Maximum biomass yields were reached by mid-June for *Alaria esculenta* (5.46 ± 0.36 kg m^{-1}) and early July for *Saccharina latissima* (5.50 ± 0.65 kg m^{-1}), although yield estimates, blade appearance, but also some compositional parameters such as calcium levels were increasingly affected by heavy biofouling, progressing from epiphytic filamentous algae to calcareous epifauna (e.g. bryozoans, barnacles) during the study. For either species, harvest times of maximum yield coincided with low moisture, high cellulose but also highest iodine contents.

Our data highlight the dramatic variability in kelp performance, considering both biomass yield and -quality, throughout the harvest season as well as the complex relationship with key environmental cultivation conditions. In addition, our findings clearly show that there is no single optimum time for seaweed harvest but rather that this highly depends on the intended application of the product. As such, we recommend harvesting early in the season for food applications (low biofouling pressure and accumulation of heavy metals and iodine, but lower biomass yield) whilst the cultivation cycle can be extended, and harvest performed late in the season to maximise biomass yields and carbohydrate contents for e.g. biofuel production.

EU-CONEXUS EUROPEAN UNIVERSITY FOR SMART URBAN COASTAL SUSTAINABILITY: EDUCATION AND RESEARCH RELATED TO AQUACULTURE

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Introduction

EU-CONEXUS is a transnational European higher education and research institution that covers the smart urban sustainable coastal development from a global point of view. It was created in the framework of the European universities' initiative (2019) including six Partners: La Rochelle Université (FR), Universidad Católica de Valencia (ES), University of Zadar (HR), Agricultural University of Athens (GR), Technical University of Civil Engineering of Bucharest (RO) and Klaipeda University (LT). European Commission selected EU-CONEXUS Plus among other 20 European University alliances, under the 2022 Erasmus+ call, supporting the rollout of the European Universities initiative for 4 more years. In this further development of EU-CONEXUS, three new partners were integrated: South East Technological University (IE), University of Rostock (DE) and Frederick University (CY). The 9 partners from all European marine regions, develop education, research and innovation projects that include the totality of social, economic, technical and environmental problems and opportunities that the coastlines are facing today. Recent EU-CONEXUS activities related to aquaculture are presented.

Materials and Methods

EU-CONEXUS offers comprehensive, joint and flexible educational activities at all levels, trying to reach out to a larger and more diverse student and staff population and foster mobility (physical, virtual or blended), multilingualism and automatic recognition of academic qualifications. Work towards the European Student Card initiative is promoted, in an effort to strengthen European identity. The Alliance implements student-centered and challenge-based approaches to education, which relies on the active engagement of students in all activities of this European University, orchestrated by the Student Board. Joint educational actions have already been developed by creating two joint Minor programmes at the Bachelor's level, as well as joint study programmes for the Master and PhD cycle. Joint actions are also regularly organized for high schools in order to raise awareness on Smart Urban Coastal Sustainability (SmUCS). EU-CONEXUS is developing a Smart Campus, a virtual pedagogical environment to help students and teaching staff of each partner institution, to share information and work together remotely, offer virtual classes and set-up an EU-Career Center. Several tools and activities have also been developed to promote EU-CONEXUS Joint Research Area and the submission of joint research projects to international calls for proposals.

Results

The EU-CONEXUS European University, launched (2021) its first joint educational offer: the "Minor in Coastal Development and Sustainable Maritime Tourism" and the "Minor in Blue Economy and Growth". Each Minor programme consists of 5 sectors; each one of them offers several courses that each student is able to select. The first graduates were awarded a Minor Certificate (30 ECTS from minimum 2 sectors, 3 different EU-CONEXUS universities, 5 courses included in the Diploma Supplement). Certain courses are provided in both semesters by different partners and teaching collaboration is promoted. For example, the course "Aquaculture" is offered to students in autumn semester by AUA and in spring semester by UCV with a similar content and certain common lectures. The Joint Master Programme in Marine Biotechnology (JMPMB), starting in September 2022, is considered as the most ambitious project activity of the Alliance. EU-CONEXUS JMPMB has been selected by the Erasmus+ programme Erasmus Mundus Joint Masters (2022-2027, Project ID: 101050597) and will be able to offer more than 20 scholarships yearly to the best applicants in 2022, 2023, 2024, 2025. JMPMB is a 2-years full time study programme, taught in English with the following structure: (a) 1st semester: core courses in Marine Biotechnology (UCV, Spain), (b) 2nd semester: core courses in Marine Biotechnology (LRUniv,

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France) and internship (at any company in any country), (c) 3rd semester: a specialisation (optional courses taught online) from 4 tracks (Innovative Bioproducts for Future; Blue Biomass; Marine Biorefinery; Aquaculture Biotechnology) and "Academic Research Integration" course where groups of students from different tracks will work together on transversal research projects (in any partner university according to the chosen track by the student), and (d) 4th semester: Master Thesis in any partner. During the 1st week of May, UCV organised several activities within the framework of European Maritime Day. A Conference was held (Valencia, 3-4 May 2022) with presentations by professors and experts from universities of the EU-CONEXUS Consortium and companies dedicated to aquaculture (AVRAMAR and CROMARIS). Students of the Aquaculture course in the Minor Programmes have been invited to UCV, to have an 'Aquaculture Week' of physical training, and carry out the blended mobility (incoming students from Greece, Lithuania and Ireland). The students attended classes and laboratory practices together and also visited the fish cages of the AVRAMAR company for learning first-hand how the fish farms operate and more about the Mediterranean aquaculture. For High Schools, the 1st EU-CONEXUS Workshop (31 March 2022) was offered virtually (in English) to Greek students (16-18 years old). Experts of two EU-CONEXUS institutions (UCV and AUA) presented and had an interactive discussion with students on topics related to SmUCS such as: (a) Coastal risk, (b) Introduction and familiarization with Fisheries and Aquaculture sectors, (c) Career in Blue Economy: Opportunities and Challenges.

The 1st physical Research Workshop of EU-CONEXUS was held in AUA (Athens, 1-2 May 2022) organized by the Research Council. The perspectives in Smart Urban Coastal Sustainability Research Workshop brought together researchers from the 9 EU-CONEXUS Universities. Vice-Rectors of Research or their delegates, presented the institutional research profiles, with more than 90 researchers attending the four parallel sessions based on the thematic areas of the Joint Research Institutes (JRIs): Coastal Engineering, Environmental Science & Biodiversity, Life Science & Biotechnology, and Social, Cultural & Human Sciences. The (AUA) as an interconnectivity portal, where users have access on researchers and staff involved in EU-CONEXUS, their affiliations, scientific interests, past and ongoing projects, research infrastructures and research teams. In order to develop EU-CONEXUS Joint Research Area, partners have started to harmonize their research protocols and processes. As a result, Multilingual Laboratory Manuals (MLMs) are being produced by EU-CONEXUS. Several protocols on analyses related to aquaculture, biotechnology and other disciplines related to marine environment have already been developed and uploaded on EU-CONEXUS Protocol Portal. External services related to aquaculture are also offered by labs, of which several are accredited or are going to be in the near future, promoting excellence and quality in research.

Conclusions

EU-CONEXUS implement challenge-based and interdisciplinary approaches to education and R&I, deepening the link with the ecosystems related to SMUCS and the broader society to tackle societal challenges, foster entrepreneurial skills and societal engagement. Research in interdisciplinary issues is promoted within JRIs, as well as networking of researchers by the EU-CONEXUS Research Portal. This European University can help the production of knowledge for the sustainable future of European coastal areas and is constantly building new bridges with the society. EU-CONEXUS offers opportunities for synergies with industry and public authorities with smart solutions on Urban Coastal Sustainability, including responsible Aquaculture. Sustainable use of marine resources, fish nutrition, carbon footprint, environmental control, consumers' perception and professional profiles are important topics for cooperation with aquaculture industry. Educating students, even from high school, about the quality of aquaculture products, eco-friendly aquaculture, ecosystem services and the social dimension of aquaculture, is a significant benefit to the fish farming value chain.

Acknowledgments

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WWF CREATING ACCOUNTABILITY IN GLOBAL FEED SUPPLY CHAINS

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WWF's progress towards removing habitat degradation from the global food system is hampered by the lack of accountability in the animal protein and feed sector which drives deforestation and conversion. We seek to gain this accountability through the development of a feed ingredient decision support tool (DST) that is fueled by a global hub of information to evaluate environmental, social and governance (ESG) risks of ingredients as well as those that supply them.

Animal protein production is the largest driver of habitat change. Habitat degradation and the over-exploitation of natural resources also are linked to human rights abuse and worker mistreatment. Thus, the production of animal protein and food creates enormous stress on both people and the planet. WWF is currently in the early stages of a global movement to eliminate habitat degradation from food supply chains. While there is progress on the slowing of habitat conversion for the rearing of farmed animals (cows, chickens, fish and swine), the habitat conversion for animal feed ingredients continues to grow because of a lack of transparency, traceability and accountability in supply chains.

The challenge of producing more food with fewer resources will require intensification of the animal protein production sector. Intensification creates a greater reliance on formulated feeds and those feeds, and the ingredients used to make them, are not under the control of producers, yet producers are held responsible for liabilities and risks that may affect their customers (retail/food service), their investors or consumers. Retailers are increasing their attention on feed related issues (see Costco and Tesco letters of support), but investors are as well (see FAIRR letter of support) because their investments in food companies may become "stranded assets" if the risk in these supply chains is not transparent. Thus, it is not only WWF that is concerned about the sustainability of feed ingredients, forward thinking animal protein producers are spotting the trends that have landed major retail chains in court.

For animal protein producers to increase production in a responsible manner, they must be equipped with the knowledge and information that allows them to identify risks – in real time - in feed ingredient supply chains as well as changing market preferences and demands. The purpose of this project is to shine the light on where, how and who produces the ingredients used in feeds for animal protein production beginning with salmon aquaculture in order to begin the transformation of the animal feed sector. We are starting with salmon because the sector has worked together to achieve common goals already. This goal is to create an ESG risk-based DST that is powered by an AI-enhanced global information hub on current impacts of ingredient production such that the most accurate and current knowledge of the current science as well as environmental, social and governance impacts of ingredient production can be shared publicly and catalyze ingredient decisions that are based on current science and values aided by transparency which disrupt the status quo. The DST will allow animal protein producers to avoid ingredients and ingredient suppliers that create the greatest impact and pose the greatest reputational and financial risks to downstream supply chain actors.

Once released, the tool will have direct application to avoiding ingredients produced by the clearing of natural habitat (legal or illegal) for row crops (Brazil, Russia, USA, etc.). The tool will also provide information on the stock status of fisheries to avoid species that are over-exploited (Chile, Norway, USA, Peru, Europe (north Atlantic fisheries). Additionally, the safeguards that are desired for workers will be on display through the transparency of policies around labor and working conditions that occur in specific countries as well as specific locales. Utilizing the human risks component of the tool will raise greater awareness on how production of ingredients can occur while providing the necessities for human well-being of workers. Fundamentally, we are seeking to have global application of the tool and create a race to the top in terms of performance such that the right actors are being rewarded for the specific attributes we expect from those who were underperformers in the past.

This project builds on existing research on protein demand changes in the developing and least developed regions of the world, as well as the patterns of climate and habitat change that will shift supply chains to different regions of the world. WWF will also merge our current efforts to decouple habitat degradation from commodity supply chains with this project to provide greater consensus around the real and perceived impacts of feed and feed ingredient production.

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The work to create greater transparency and accountability in the animal feed sector is paramount for the sustainability of food systems. Without this accountability, feed ingredients will remain in the shadows and continue to consume a tremendous amount of the planet's resources. To our knowledge, this is the first effort to truly confront these issues with a lens to promote greater stewardship in resources used through the production of animal feed. The sustainability of animal feed is one of the greatest challenges for our food system with direct implications for the emissions that contribute to climate change, the conversion of our natural ecosystems that buffer humans from climate change and zoonotic diseases and the protection of the biodiversity in habitats threatened by conversion.

COMPARING OPPORTUNITIES AND CHALLENGES IN PRODUCTION SYSTEMS FOR SALMON AQUACULTURE

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Introduction

Norway is a leading producer of Atlantic Salmon (*Salmo salar*) on a global scale. However, growth in production in conventional sea-based fish farming is curbed by challenges such as salmon lice and escape of fish. The industry is eager to innovate to solve these challenges. Policies to develop new concepts for aquaculture has also been initiated by the government (Føre et al. 2022). Currently, the Norwegian salmon aquaculture industry is characterized by rapid development, establishment and operation of new production systems including land-based, floating closed and semi-closed and open ocean aquaculture systems.

Regulation and localisation of new production systems requires knowledge about the systems. Applying a comparative perspective, the objective of this study is to provide knowledge of industry actors perceptions of challenges and opportunities related to new aquaculture production systems in Norway. This knowledge is valuable for a sustainable development and regulation of the industry.

The project “Compareit” is financed by the Research Council of Norway (grant number 319647). It is a *collaborative project to meet societal and industry-related challenges* conducted in close collaboration with key industry actors in the Norwegian aquaculture industry.

Materials and methods

To explore perceptions of industry actors, interviews, workshops, and document studies have been key methods. Interviews and workshops related to the following research questions: What are considered key challenges and opportunities within each production system? What are considered key challenges related to regulation of new concepts?

Results

For all new systems, the opportunity of production growth is seen as a key motivation. For the different systems, certain aspects are highlighted to obtain this in a sustainable manner. Land-based systems are designed to increase bio security (less patogens and contamination), and farmers aim to collect and utilize the organic material to create circular economy. Still, the industry required to process waste needs to be established to reach this ambition. Looking at challenges, disinfection of the water and formation of hydrogen sulphide are mentioned. The technology is considered complex, and energy use is potentially high.

Semi-closed and closed systems in the sea also require more energy than conventional sea-based fish farms to pump water into the systems, but these systems allow for using areas that are not suitable for conventional open net pens that are more sheltered in fjords etc. Furthermore, the opportunity to prevent sea lice and escapes are important drivers for this technology.

For open ocean aquaculture systems, key opportunities include utilizing new areas, better water quality, stable temperatures, and water exchange. Industry actors state that there is a need to acquire more knowledge about the challenges related to fish health and welfare in open ocean locations.

For new production systems, regulators must handle new issues and industry actors express that some of the processes are time-consuming and unpredictable. In the case of open ocean aquaculture for instance, the areas in question fall outside the responsibility of the municipalities, and the government are currently working on a new regulatory regime that aims to facilitate for production in new areas.

Conclusion

This study shows that both opportunities and challenges exist in different production systems for Atlantic Salmon in Norway. What the industry may look in the future depends on solving these challenges and establishing regulation that contributes to sustainable production for all production systems.

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COST EXPLOSION IN SALMON AQUACULTURE: THE IMPACT OF DISEASES, PARASITE INFESTATIONS AND REGULATIONS

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Since the inception in the early 1970s, salmon aquaculture production has exhibited very high growth rates. However, in the last decade growth rates have fallen dramatically. Authorities' concerns with negative externalities from salmon farming arising from the impact of sea lice, diseases and escapees on wild salmon have led to stricter environmental regulation and reduced production growth. While numerous studies have examined the impact of sea lice and diseases on wild salmonids, very few studies have addressed the private costs of sea lice and diseases. The same environmental factors affecting wild salmon also have a negative impact on cost and fish welfare for farmed salmon. Over the last 15 years, production costs have increased by nearly 150 percent, and 40 percent just over the last 5 years. Using firm level data, we analyze the impact on cost variation from diseases, parasite infestations and regulations. Our results show that since 2012, cost heterogeneity has increased dramatically, and can empirically be attributed to increased private costs of sea lice infestations, suggesting that stricter regulation has resulted in an internalization of externalities from salmon aquaculture.

THE EFFECTS OF GARLIC (*Allium sativum*) AND HENNA (*Lawsonia inermis*) EXTRACT ON REDUCING THE FUNGAL LOAD OF FERTILIZED AND EYED EGGS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Green malachite is one of the aniline-colored products and is known to be mutagenic and carcinogenic. The use of this chemical compound in fish intended for human consumption has not been approved by the US Food and Drug Administration. Following the application of restrictions on the use of green malachite due to its very adverse environmental effects and the complete safety of garlic and henna plant extracts, this material was investigated as an alternative to green malachite.

This research was done in a completely practical way and in environmental conditions. In this method, five trays were selected, three trays with plant extract and two trays as control with green malachite and pure water with temperature conditions of 13 degrees Celsius with pH 5.7 and oxygen solubility of 6 mg/liter for 17 days.

From the green stage to the emergence of the egg with concentrations of 25, 50 and 70 mg/liter in the treatments with the extract and 3, 5 and 10 mg/liter in the green malachite treatment each with 3 repetitions by daily washing method for 1 hour was tested.

The results showed that the combined use of two extracts of garlic and henna with a concentration of 70 mg/liter had 70% survival in the egg hatching stage and based on statistical data, it had a significant difference with the samples and concentrations of the control treatments. It can be a suitable substitute for chemical disinfectants such as green malachite.

IDENTIFICATION OF THE MICROFLORA OF THE GASTROINTESTINAL SYSTEM OF RAINBOW TROUT (*Oncorhynchus mykiss*) AND THEIR POSSIBLE IMPORTANCE IN TERMS OF PROBIOTICS

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Today, the role of the microflora of the digestive system in maintaining the health and comfort of animals is undeniable. The present study was carried out with the aim of identifying the microflora of the digestive system of rainbow trout and their possible importance in terms of probiotics. For this purpose, 20 rainbow trout fry weighing 2 grams were prepared and provided to the project, and during the growth period, disinfectants and antibiotics were not used. After the fish reached the desired weight, they were transferred to the laboratory and biometrically measured, and they were in the range of weight between 3010 grams and total length of 8 to 12 cm. After disinfection of fish with 70% alcohol and dissection with sterile tools, external intestine and preliminary and differential microbial culture including culture with Nutrient Agar, MacConkey Agar, TSI, TSA, SIM, MR-VP, Urease Test Broth, E.M.B, Starch media. Agar was done. Gram staining was used to identify bacteria. Also, biochemical tests and reagents including methyl red, Vojsprosquare-barite reagent, Coax reagent, oxidase-catalase test, sugar dough test in OF environment, gelatin decomposition test and starch hydrolysis test were used for definitive diagnosis of bacteria.

The results of this research indicate that the bacterial flora and their percentage in rainbow trout include *Edwardsillate sp.* (8%), *Cirobracer sp.* (10%), *Shewanella sp.* (30%), *Hawmophilus sp.* (2%), *Plesiomonas sp.* (25%), *Aeromonas sp.* (25%). Predominant flora with probiotic potential includes *Shewanella sp.*, *Plesiomonas sp.* and *Aeromonas sp.*

THE GROWTH POTENTIAL AND QUALITY OF MEAGRE (*Argyrosomus regius*) AT WARMER TEMPERATURES, A CASE STUDY FROM THE RED SEA

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Introduction

Meagre is a widely diffused species that thrives in the Mediterranean but also in the Atlantic Ocean, from north Europe to equatorial Africa. The optimal texture and taste as well as its fast growth and environmental adaptability makes it an ideal candidate for aquaculture exploitation. The production of meagre, which occurs mainly in the southern part of the Mediterranean Sea, outlines how better growth performances can be achieved in warmer temperatures and in areas with milder winters. The scope of this research was to determine the growth potential, feed response and the body characteristics of meagre reared in warmer seawater, such as the Red Sea environment.

Materials and Methods

Two different trials involving juveniles of 115 g (Run 1) and pre-adults of 568 g (Run 2) were carried out in order to assess the growth potential of *Argyrosomus regius* at different growth stages. The fish in both trials were stocked in 1.1 m^3 tanks, with 41 juveniles stocked in triplicates per tank (4 kg m^{-3}) while pre-adult were stocked in number of 16 individuals (8 kg m^{-3}). Fish were fed twice a day for a period of 74 days. Four locally available commercial diets were used, with values of crude proteins and crude fat (CP/CF) for juveniles as follows: 47/14 (Diet 1), 46/13 (Diet 2), 47/12 (Diet 3) and 42/20 (Diet 4). For pre-adults the CP/CF values were similar to the juvenile trial, with the only exception of Diet 2, whose CP/CF was 48/14.

The water salinity in both trials was 41-42 g L^{-1}, while temperature was 25.32 ± 1.21 °C in Run 1, and 27.34 ± 0.7 °C in Run 2.

Fish data was analyzed using one-way ANOVA followed by Fisher Least Significant Difference (LSD) test to compare significant differences between treatments.

Results

The juvenile fish grew between 130 to 209 g during the 74 days of feeding. Final body weight was statistically higher in Diet 1 (324g) than in the other ones (range 295-246 g). Specific growth rate (SGR) resulted highest in Diet 1 with 1.4 ($P < 0.05$), medium in Diet 4 (1.25) and lowest in Diets 2 and 3 (1.00 and 1.04 respectively). The FCR was higher in Diets 1 and 4 (1.09 and 1.18) than Diet 2 and 3 (1.52 and 1.41).

The final body weights of pre-adult fish resulted highest in Diets 1 and 2 (770 g and 733g). Similarly SGR for the same diets were respectively 0.40 and 0.37, higher than Diets 3 and 4 (0.17 and 0.14). The FCR was not statistically different in Diets 1 and 2 (1.61 and 1.85).

The protein efficiency ratio in juveniles was statistically higher in Diet 1 and 4 (1.82 and 2.17 respectively), and in Diets 1 and 2 for pre-adult (1.33 and 1.08).

Proximate composition of the body of pre-adult fish showed no differences in crude protein % but showed higher level of crude fat % in Diets 2 and 4.

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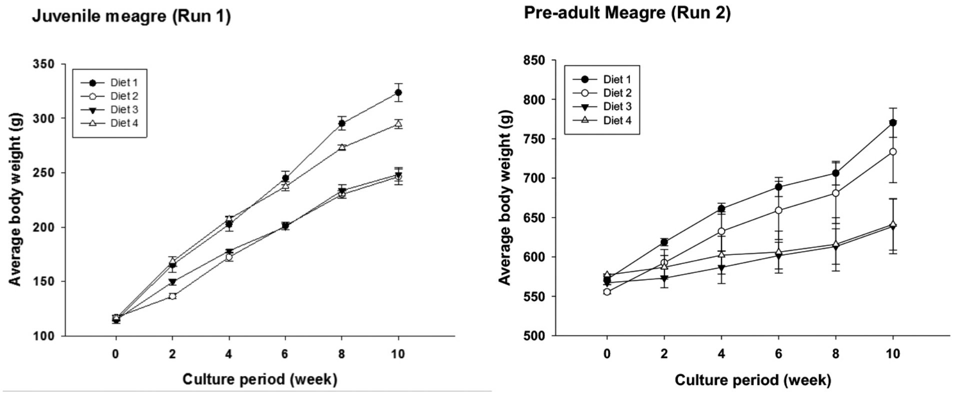

Fig. 1. Average body weight of juvenile and pre-adult meagre fed the different commercial diets. Values represent means ± S.E.M. (n=3)

Discussion

The current study showed that the growth performances of meagre were comparable to other researches carried out at lower temperatures or salinities. In Run 1 the performances were comparable to the FCR of 1.2 obtained by Martínez-Llorens et al., (2011) using 47% CP and 20% CF. In juveniles the SGRs were similar or higher than the values found in the literature. In pre-adults the FCR showed similar or even better results than most of the papers with same body weights. However SGRs were slightly lower than the 0.57 obtained by Gültepe et al. (2016) or the 1.77 obtained by Velazco Vargas (2014). The proximate concentration of the carcass of the fishes showed higher percentages of proteins compared to the 15.8-16.7 % of Carvalho et al. (2018).

Conclusions

Meagre tested in the Red Sea conditions proved comparable growth with all the scientific researches carried out in the Mediterranean Sea, particularly for juvenile fish. Some suboptimal performances in pre-adult fish could be motivated by the higher temperatures, the small size of the pellets as well as some nutritional flaws determined by the use of commercial feeds not specifically formulated for meagre.

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DIFFERENT METHODS OF OVULIN HORMONE INJECTTION ON STREES INDICIES (GLUCOSE AND CORTISOL) OF CASPIAN CARP (*Cyprinus carpio*)

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Introduction

Glucose and cortisol are important indicators in assessing the level of stress in living organisms. Blood glucose is the most variable index that is greatly affected by handling, transportation, environmental stress, seasonal changes, nutritional status and maturity (Hosseini Fard et al., 2013). Cortisol in bony fish affects reproductive function and gamete growth. Cortisol, an important corticosteroid produced during the spawning season, can suppress fish immunity (Odhiambo et al., 2020). Inducing sexual maturation in fish can be stressful and can have an adverse effect on fish reproduction. Hormone injections into fish for the purpose of inducing reproduction, in addition to affecting the levels of sex steroids, can cause changes in fish's blood stress indices (Falahatkar et al., 2016). Therefore, it is important to use a method that puts the least stress on the fish. In this study, different methods of injecting the Ovulin hormone on glucose and cortisol of Caspian carp were studied.

Materials and Methods

12 Caspian carp were injected in three methods in one time: injection under the pectoral fin, injection under the dorsal fin and injection under the ventral fin at a dose of 0.6 ml / kg of Ovulin hormone. Blood samples were taken from male and female broodstocks with 5 cc syringes and from fish tail arteries in two stages (before hormone injection and 12 hours after hormone injection). In the hematology laboratory and after centrifugation at 5000 rpm, serum was isolated from blood cells for 5 minutes and glucose and cortisol levels were measured by automated devices. All statistical analyzes were performed using SPSS software version 26. Shapiro-Wilk test was used to investigate the normal distribution of data in groups and for all variables. One-way analysis of variance (Oneway ANOVA) was used for statistical comparison between groups, and after performing the Test of Homogeneity of Variances and Duncan test was used to compare groups with each other (Mohammad Nejad, 2022).

Results and Discussion

The results of glucose and cortisol analysis in male and female broodstocks of Caspian carp showed that in both sexes and in all three injection methods, its amount increased after hormone injection (Tables 1 and 2).

The amount of stress increase due to hormone injection in both male and female broodstocks in the method of pectoral fin and dorsal fin was more than the method of injection under the ventral fin. The comparison between the two broodstocks also showed that the increase in stress in the female broodstocks was more than the male broodstocks. These results were consistent with the study of changes in stress indices of *Cyprinus carpio* in different stages of artificial reproduction (Mohammad Nejad, 2022). The results of this study show that hormone injection is effective in increasing stress in Caspian carp. Consistent with the results of this study, injection of various hormones in artificial reproduction increased glucose and cortisol levels in some fish (Mohammad Nejad, 2022). Almost any type of stress (physical and neurological) causes an immediate and pronounced increase in ACTH secretion and within minutes leads to a sharp increase in cortisol secretion from the cortical part of the adrenal glands. One of the many effects of cortisol is to increase the body's resistance to stress by reducing glucose uptake, so as cortisol increases, blood glucose levels also increase rapidly (Saha et al., 2003), which was also proven in the current study. Due to the injection of Ovulin hormone in male and female broodstocks, the amount of cortisol increased due to stress and then the absorption of glucose from the blood decreased and as a result, the amount of glucose in the blood serum increased.

However, the relationship between stress and reproductive hormones of Caspian carp was not investigated in the present study but, the relationship between stress and its indicators with sex hormones in fish has been proven. The effect of cortisol on reducing estradiol and testosterone has been reported in some fish (Falahatkar et al., 2016). Therefore, using a method that can put the least stress on the fish during the breeding process is important and necessary. The results of the present study show that hormone injection by injection method under the dorsal fin can cause less stress to the Caspian carp, so it is recommended compared to the other two methods in the reproduction of this fish.

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Table 1: Stress indices of female broodstocks of caspian carp in different methods of Ovulin hormone injection

Stress index	Injection area	Before injection	After injection
Glucose	Pectoral fin	79.5 ± 5.5^{a}	$125 \pm 4^{a*}$
	Dorsal fin	72.5 ± 4.5^{a}	$113.5 \pm 4.5^{a*}$
	Ventral fin	68 ± 6^{a}	$108.5 \pm 5.20^{a*}$
Cortisol	Pectoral fin	8.84 ± 0.43^{a}	$114.5 \pm 7.5^{a*}$
	Dorsal fin	7.88 ± 0.47^{a}	$21.25 \pm 1.15^{b*}$
	Ventral fin	9.39 ± 3.84^{a}	$108 \pm 4^{a*}$

Non-common Latin letters for each index in each column indicate the difference between the injection methods and the * in the row indicates a statistically significant difference before and after the injection ($p < 0.05$).

Table 2: Stress indices of male broodstocks of caspian carp in different methods of Ovulin hormone injection

Stress index	Injection area	Before injection	After injection
Glucose	Pectoral fin	49.5 ± 3.5^{a}	$91 \pm 3^{ab*}$
	Dorsal fin	55.5 ± 4.5^{a}	$121 \pm 5^{a*}$
	Ventral fin	48.5 ± 6.5^{a}	$83.5 \pm 10.5^{b*}$
Cortisol	Pectoral fin	16.15 ± 0.75^{a}	$27.7 \pm 0.5^{a*}$
	Dorsal fin	13.55 ± 0.77^{a}	17.95 ± 3.25^{b}
	Ventral fin	9.03 ± 0.89^{b}	$28.8 \pm 1.4^{a*}$

Non-common Latin letters for each index in each column indicate the difference between the injection methods and the * in the row indicates a statistically significant difference before and after the injection ($p < 0.05$).

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EVALUATION OF ENZYMATIC TREATMENT OF PLANT PROTEINS AND NATURAL NUTRACEUTICALS IN AQUAFEEDS OF GREATER AMBERJACK (*Seriola dumerili*) JUVENILES

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Introduction

With the continuous growth of the human population and the increase in per capita fish consumption (FAO, 2022), aquaculture represents a great opportunity as a global food source, being a solution to mitigate overfishing. One of the problems in the aquaculture industry is the use of fish meal and fish oil as sources of proteins and lipids for feeds, especially in carnivorous species such as greater amberjack (*S. dumerili*). Therefore, it is necessary to look for alternative ingredients to make their farming more sustainable and, perhaps, more profitable. Considering that feed in the aquaculture industry accounts for around 60% of production costs (APROMAR, 2022), the use of plant sources as raw materials is not only interesting from sustainability, but also from profitability. However, in carnivorous species, the use of plant sources can have adverse effects due to lower digestibility, so a solution could be the use of biotechnological treatments to increase the bioavailability of their nutrients, as has been demonstrated with microalgae (Agboola et al., 2019; Molina-Roque et al., 2022). Furthermore, the benefit of including nutraceutical compounds in the diet has been demonstrated on numerous studies (Dawood et al., 2015; Kader et al. 2013; Perera et al., 2020), so the addition of this type of ingredient in the diet of carnivorous species could mitigate the possible adverse effects of using plant sources.

Material and Methods

A total of 225 individuals (initial body mass, ~ 6 g) were distributed in 9 tanks of 400 L, which constituted the 3 experimental groups (in triplicate): i) Control, fed with standard formulation; ii) PP, feed with 50% protein from plant sources enzymatically treated to increase their bioavailability; iii) PP-LB, similar to the PP diet, but with the inclusion of 3% nutraceutical compounds. After 69 days of *ad libitum*-feeding trial, a final biometric sampling was performed, and samples from plasma, liver and intestine were taken. Somatic and zootechnical indices were calculated, and samples from each tissue (plasma and liver metabolites, circulating cortisol levels and intestinal resistance as a measure of intestinal integrity) were analysed.

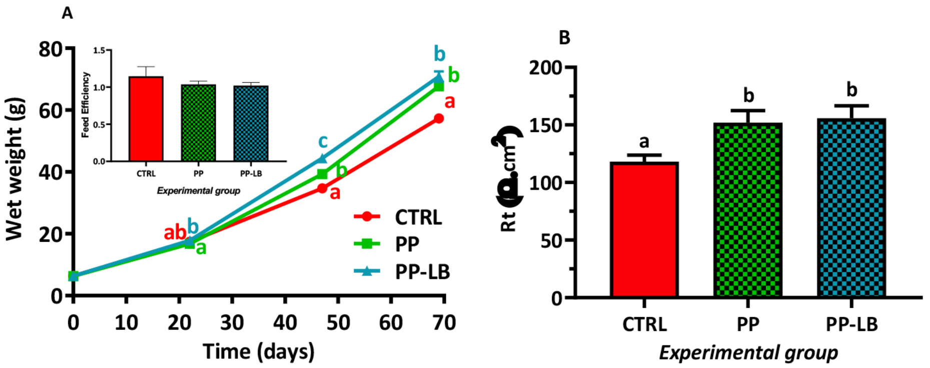

Fig. 1. Evolution of the wet weight and feed efficiency (**A**), and intestinal epithelial resistance (**B**) of *S. dumerili* juveniles fed with the three diets. The results are expressed as the average ± SEM (n = 12 fish). Different letters represent statistically significant differences at a p-value<0.05 resulting from the one-way ANOVA analysis.

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Results and Discussion

The results indicated an increase in growth performance with the two experimental diets (PP and PP-LB) associated with a stimulation of fed intake, gut elongation and favourable metabolic orchestration, despite a slight increase in circulating cortisol levels. In fact, the moderate presence of cortisol stimulates the production of neuropeptide Y (NPY), which promotes feed intake (Bernier et al., 2004) and increases absolute metabolic scope (Pfalzgraff et al., 2022), which would support the results observed about growth performance with the two experimental diets. Furthermore, with the use of electrophysiological analysis of the intestine, a significant increase in epithelial resistance has been observed, resulting in increased selectivity and integrity of the intestine in the specimens fed with the two experimental diets, and demonstrating a good intestinal health.

All these results demonstrate an improvement in nutrient assimilation and utilisation, which leads to a higher energy investment for somatic growth, indicating that the use of pre-treated plant-based raw materials is optimal in a high-growing carnivorous species.

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IMPROVED FERTILIZER MANAGEMENT FOR SMALL TO MEDIUM SIZED COMMERCIAL DECOUPLED AQUAPONIC SYSTEMS

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Introduction

The potential of becoming one of the most effective sustainable production systems for the combined production of animal protein and plant crops is attributed to decoupled aquaponic systems (Kloas et al. 2015, Monsees et al. 2017). Here, recirculating aquaculture systems for fish production are combined with hydroponic systems for soilless plant production allowing individual management of each single compartment thereby recycling dissolved nutrients derived from metabolism of the fish. Nevertheless, professional adjustment of selective micro- and macro-nutrients can be very expensive, especially for small to medium aquaponic operations (e.g. complete lab analysis of RAS-derived water). Therefore, (i) one of the aims of this study is to provide cost effective strategies for nutrient management in aquaponics systems. In order to meet specific plant requirements, the knowledge on nutrient concentrations as well as predictable patterns and trends in the RAS water is pertinent for correct fertilizer addition. Consequently, further aims are to (ii) identify the origins of macro- and micro-nutrient and to find accumulation patters, as well as the attempt to predict their general trends, and nutrient dynamics (N,P,K), (iii) to meet plant needs as close as possible according to a professional fertilizer management in hydroponic production units.

Material & methods

Literature values from RAS and aquaponic applications were collected with regard to predefined boundary conditions. Additionally, water samples were collected from different research or commercial RAS or corresponding aquaponic facilities. Micro- and macro-nutrients were analyzed using continuous flow analysis (CFA) and inductively coupled plasma-optical emission spectrometry (ICP-OES). Models were tested in a first trial with two different treatments against a hydroponic control (n = 3, 10 lettuce plants per replicate).

Results

General nutrient patters for RAS and aquaponic facilities were identified and used for the development of models. The first results of the model testing in a hydroponic setup revealed that lettuce can be effectively produced in an aquaponic setup without the need for detailed nutrient analyses. The lettuce growth was comparable between both aquaponic applications, but significantly reduced by 10 % compared to the hydroponic control (Fig. 1).

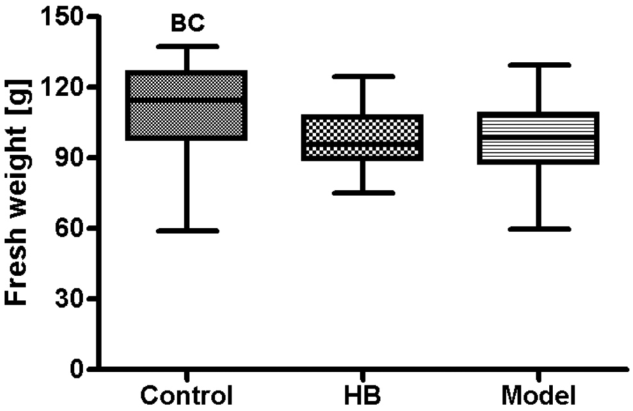

Fig. 1. Lettuce growth in three different hydroponic treatments. C = control, tab water based nutrient solution; HB - aquaponics, fish water based nutrient solution with supplemented nutrients after detailed nutrient analyses; Model - like HB, but without detailed nutrient analyses prior to nutrient supplementation. The three different treatments were applied in triplicates, each with ten individual lettuce plants.

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Discussion

The potential of identifying and using nutrient patterns in RAS and aquaponics for a combined production of fish and plants in a decoupled aquaponic approach were clearly shown in this study. By applying this easy approach, professional nutrient management can be simplified for small and medium aquaponic producers. The first results are very promising, showing that overall aquaponic yield is not affected with respect to the type of nutrient management. The post adjustment of the nutrient profile using fish water for professional hydroponic application was more challenging as in conventional hydroponics (with rain water or tap water) but it was demonstrated that for most nutrients the set points were reasonably close to the recommended nutrient concentrations as it was also shown in other studies (Suhl et al. 2016, Monsees et al 2019).

Additionally, the authors are very optimistic that with ongoing professionalization and standardization of practices in decoupled aquaponic technology, more farmers will adapt towards professional management approaches and that comparable yields to conventional hydroponic production can be expected, as it was already show e.g. in Monsees et al. 2019.

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OTOLITH SHAPE: A TRACER FOR AQUACULTURE FISH SPECIES?

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Introduction

Among the impacts produced by the aquaculture industry, fish escapes are considered one of the most harmful in terms of wild population unbalances. Developing reliable traceability tools becomes essential to assess escape events extent and ecological consequences. Bio-morphological analysis have been extensively used to differentiate geographically separated populations as well as wild stocks from aquaculture counterparts. Former studies made use of body shape and solid structures such as scales and otoliths to determine fish origin [**1**]. Somatic growth patterns are reflected in these structures by means of mineral layer depositions, which are known to be related to life phases, seasons, and habitat transgressions. Nonetheless, little is known about how they adjust their morphology through feralisation processes, meaning a potential bias for stock identifications. Considering the solid background of otolith growth rings and microchemistry as traceability markers, the present study explores differences between wild, farmed and escaped fishes according to otolith external macro and microstructure.

Material and Methods

We collected 131 sea breams (*Sparus aurata*), 135 sea basses (*Dicentrarchus labrax*) and 76 meagres (*Argyrosomus regius*) from fish markets located in the Spanish East Coast (C. Valenciana and Murcia regions). Based on body shape analyses [**1**], fish distribution by origin was established (Fig.1-A). After labelling, weighting, and taking biometrics, we performed dissections and extracted otolith larger fractions (pair of *sagittae*). Once clean and dry, we took bird-eye pictures of the left *sagittae* piece, placing the *sulcus acusticus* (groove) face upwards (Fig.1-B). We enhanced otolith shape by increasing the contrast with reflected light. Resulting pictures were processed in two diverse ways: 1) univariate morphological descriptors (uMDs) measurement (area, perimeter, roundness, circularity, axis length and aspect ratio); and 2) Fourier analysis based on Elliptic Fourier Descriptors (EFDs) [**2**]. Briefly, it decomposes two-dimensional closed silhouettes into a set of ellipses or EFDs. Using applied software such as SHAPE, EFDs can be transformed into numerical coefficients (a, b, c, d) that, in turn, can be used to compare the shape of two or more sets of figures.

Prior to statistical analysis, we removed allometry effect over data from uMDs by Burnaby vs Standard normalization. Regarding EFDs normalization, we prevented any figure orientation or rotation angle effect over the analysis by subtracting the first 3 coefficients from the first EFD of each sample. To check normalization effectiveness, we used a data resemblance matrix by Euclidean distance and performed PERMANOVA analysis with length (TL) as covariable ($p < 0.05$; perm: 9999).

Searching for morphological divergence between otoliths from origin groups (Wild (W), Farmed (F) and Escaped (E)), we conducted several statistical tests: 1) Principal Component Analysis and 2) Discriminant Function Analysis based on uMDs and 3) Principal Component Analysis using EFDs coefficients.

Results

Principal component analyses allowed us to visually separate origin groups in different data clouds, enclosed within 3 ellipses (Fig.1-C). Both, uMDs-based and EFD coefficients-based, displayed the same tendency: the three ellipses overlapped to a greater or lesser extent. However, farmed and escaped groups showed a more similar data dispersion range compared to wild. Otoliths from wild individuals tend to reflect a lower diversity regarding shape, especially in *S. aurata*. Focusing on microstructure, PCAs link *S. aurata* otoliths morphological variations to changes in ostium opening, post-cauda angle and overall roundness. *D. labrax* otoliths mainly differ in post-cauda and rostrum angle. *A. regius* displays slight differences in post-ventral margin roundness and lateral irregularities. DFA displayed precision rates from 50 to 77%, a low accuracy.

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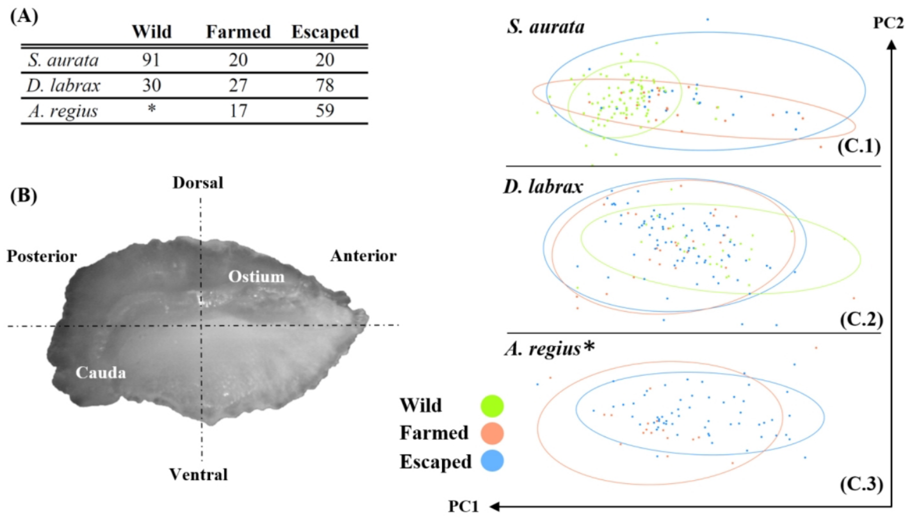

	Wild	Farmed	Escaped
S. aurata	91	20	20
D. labrax	30	27	78
A. regius	*	17	59

Fig. 1. A: Sample distribution by species/origin. *No evidence of wild *A. regius* populations in the studied area; B: Sea bream otolith regions/structure; C: PCA based on Elliptical Fourier Descriptors for wild, farmed and escaped groups.

Discussion

Overall, there were no significant differences between wild, farmed and escaped fishes in regard to otolith external morphometry. Supporting former literature [**1**], otolith shape should only reinforce scales or body morphometrics. However, data dispersion and overlapping degree among groups provided insightful information. On their journey to the wildness, escapees are subjected to stressors such as lack of resources or shifts in social and physical conditions. Success or fail depends on a balance between phenotypical plasticity and selective pressure. By contrast, aquaculture farms ensure survivability by granting abundant/constant food resources, lack of predators, disease control... favouring wider otolith shape diversity. Moreover, otolith macro- structure appears to be resilient trough feralisation over extended periods. Last, we have identified species-specific regions susceptible to suffer microstructural changes. We are currently balancing our data and exploring fish origin effects on otolith long-term development.

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REPLACING FISH OIL BY SUSTAINABLE MARINE LIPID SOURCES IN DIETS FOR TURBOT (*Scophthalmus maximus*): EFFECTS ON GROWTH PERFORMANCE, NUTRIENT BALANCE AND MUSCLE FATTY ACID COMPOSITION

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Introduction

Fish oil (FO) is the ideal lipid source for carnivorous marine fish diets due to its high digestibility and high essential fatty acids content, particularly long-chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Given FO limited supply, sustainability issues, and increasing costs, alternative lipid sources must be found. Replacing FO is not a simple task as often the alternative sources modify tissue fatty acid (FA) composition and may compromise the nutritional benefits associated with fish human consumption. Thus, in the present study, the potential of sustainable marine lipid sources was evaluated in turbot (*Scophthalmus maximus*) focusing on the dietary effects on growth performance, nutrient balance, and body FA composition.

Materials and methods

Six extruded isoproteic (60% dry matter - DM), and isoenergetic (22 kJg^{-1} DM) diets were formulated (Table 1). The control diet (CTRL) included FO (sardine oil) and rapeseed oil as the main lipids sources and was formulated to include 2.7% EPA+DHA. The other five diets included different marine lipid sources to partially and totally replace FO and fortify turbot muscle in omega-3 LC-PUFA, without major increase in production costs: algae oil (ALGASARDINE), salmon oil (a co-product from the salmon industry) and a blend of microalgae (ALGAESSENCE). A FO-based diet was also included as a positive control (SARDINE). In ALT1 and ALT2 diets, FO was totally replaced by salmon oil and algae oil and fishmeal was replaced at 30% by *Tenebrio molitor* meal and a protein concentrate from *Corynebacterium glutamicum*. Fish (150 g) were reared in a commercial farm (~15 °C) and fed twice a day at apparent satiation. After 75 days, growth performance, feed efficiency and total lipids and FA profiles in whole body and muscle were determined.

Results

All groups of fish almost doubled their initial body weight and there were no differences in growth performance and feed utilization amongst dietary treatments. FO replacement had a clear effect on FA muscle deposition. EPA gain was highest in fish fed CTRL, SARDINE and ALGAESSENCE diets, while docosahexaenoic acid (DHA) gain was highest in the ALT1 group. No significant differences were observed in EPA+DHA retention. All experimental groups presented low muscle lipid levels (< 1%) and similar EPA+DHA levels. Fish fed ALGAESSENCE were the only ones able to surpass EFSA recommended EPA+DHA levels for human consumption (275.5 mg 100g^{-1}); ALT2 group exhibited values equivalent to SARDINE (~ 230 mg 100g^{-1}) and for the remaining dietary treatments values of 215 mg 100g^{-1} were obtained.

Table 1 – Protein, lipid sources, expected EPA+DHA and estimated cost of the experimental diets

	CTRL	**SARDINE**	**ALGASARDINE**	**ALGAESSENCE**	**ALT1**	**ALT2**
Ingredients (%)						
Fishmeal LT70	55.0	55.0	55.0	55.0	38.5	38.5
Tenebrio meal	-	-	-	-	7.0	7.0
C. glutamicum	-	-	-	-	2.5	2.5
Sardine oil	7.0	10.7	5.4	9.5	-	-
Salmon oil	-	-	-	0.7	8.0	6.0
Algae oil	-	-	2.7	-	2.9	4.9
Rapeseed oil	3.8	-	2.7	-	-	-
Blend of algae	-	-	-	2.0	-	-
EPA+DHA	**2.7**	**3.7**	**3.7**	**3.7**	**2.7**	**3.7**

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Discussion and conclusions

Replacing up to 100% of fish oil with the alternative lipid sources did not significantly affect fish growth performance or feed utilization in *S. maximus*. All fish presented similar muscle EPA+DHA values, but only the ALGAESSENCE fed group reached values above EFSA recommendation (275.5 mg $100g^{-1}$) for preventing cardiovascular diseases. This suggests that the combination of alternative sources of lipids, and particularly the inclusion of an algae blend (ALGAESSENCE) may be a viable alternative for replacing FO in turbot diets.

This trial was carried out in juveniles under commercial conditions often variable and difficult to control. In order to carry out further evaluation of the impact of diets with different fatty acid compositions, the use of an energy-protein flux model coupled with a fatty acid dilution sub-model is envisaged, which will allow us to predict fish growth and muscle LC-PUFA deposition for different scenarios. This tool will help defining best strategies and finishing diets adapted to a particular farm.

Acknowledgements

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EXPLORING THE POTENTIAL OF LOW TROPHIC SPECIES (*Alitta virens*) TO REPLACE FISH MEAL IN DIETS FOR EUROPEAN SEA BASS

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Introduction

The aquaculture industry and scientific community have been trying to shift to a lower usage of marine-harvested resources, such as fish meal, in aquafeed production. Low trophic marine species, like Polychaeta, can contribute to practices able to support a sustainable blue economy. They are potential candidates for bioremediation of aquaculture waste and may be a viable alternative protein source to fish meal due to its high protein content and high levels of ω3 LC-PUFA. They are also rich sources of bioactive compounds able to modulate fish immunological system and/or stress response.

The overall aim of this study was to assess the potential of Polychaeta meal to replace fish meal in European seabass diets assessing growth performance, nutrient utilization, whole-body composition, muscle fatty acid profile, and liver lipid metabolism.

Materials and Methods

A growth trial with European sea bass (*Dicentrarchus labrax*) juveniles (14.5 g) was performed using four isoenergetic (22% dry matter, DM), isoproteic (51% DM), and isolipidic (17% DM) diets. A fish meal-based diet (FM, control) was compared with three experimental diets including 2.5% (PM2.5), 5% (PM5) and 10% (PM10) of spray dried Polychaeta meal (PM, *Alitta virens*), to replace 10%, 20% and 40% of FM, respectively. All experimental diets were extruded by Nofima (Norway). Triplicate homogeneous groups of fish per treatment were divided by twelve 160 L fiberglass tanks and fed by to satiety using temporized automatic feeders, three times a day (9h, 12h, and 16h), for 93 days. The fish were subjected to a 12-h light/12-h dark photoperiod regime and kept in a recirculating saltwater system (salinity 35‰, 22 ± 1 ºC).

At the end of the growth trial, all fish were individually weighed and measured. Five fish per tank were sampled for whole-body composition; other 6 fish were sampled to collect dorsal muscle for total lipid and fatty acids analysis and liver samples for analysis of lipogenic enzymatic activity. The remaining fish were used for a digestibility trial to evaluate the apparent digestibility of crude protein, crude fat, and gross energy in the diets using yttrium oxide as an inert marker.

Table 1 – Growth performance and feed intake of European sea bass fed the experimental diets for 93 days. Values represent the mean ± standard deviation.

	Diets			
	FM	PM10	PM20	PM40
IBW	14.47 ± 0.03	14.52 ± 0.01	14.49 ± 0.02	14.52 ± 0.01
FBW	76.67 ± 0.23	78.07 ± 1.60	79.10 ± 3.80	77.77 ± 3.01
K	1.17 ± 0.03	1.21 ± 0.01	1.19 ± 0.04	1.19 ± 0.01
DGI	1.95 ± 0.01	1.97 ± 0.03	1.99 ± 0.07	1.97 ± 0.06
VFI	1.54 ± 0.07	1.54 ± 0.03	1.55 ± 0.03	1.52 ± 0.07
FCR	1.05 ± 0.04	1.05 ± 0.02	1.04 ± 0.01	1.03 ± 0.03
PER	1.84 ± 0.07	1.89 ± 0.03	1.87 ± 0.02	1.90 ± 0.06

IBW– initial body weight (g); FBW – final body weight (g); K – final condition factor; DGI – daily growth index; VFI – voluntary feed intake; FCR – feed conversion ratio; PER – protein efficiency ratio.

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Results

All fish accepted very well all experimental diets. Replacement of FM by PM had no effect on the voluntary feed intake, daily growth index (2.0), or feed conversion ratio (1.0) of the fish (Table 1). Hepatosomatic and viscerosomatic indexes also remained similar among treatments. Nutrient retention and gain, flesh fatty acid profile, and liver lipid metabolism results will be further analyzed and discussed.

Discussion and Conclusion

The present results indicate that PM can successfully replace FM up to 40%, without impairing feed intake, growth performance, and nutrient utilization. Thus, this invertebrate marine biomass seems a viable new ingredient for sea bass diets, replacing fish meal and contributing to food security. However, the functionality of Polychaeta meal remains to be explored.

Acknowledgments

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SALINE WASTEWATER FROM FISH AND OYSTERS TO GROW DIFFERENT SPECIES OF HALOPHYTES IN GREENHOUSE

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The rapid growth of the aquaculture production over recent years has led to a significant increase in marine wastewater being discharged to natural ecosystems following the use of seawater in fish production. This waste effluent contains high concentrations of nitrogen, phosphate and nitrate and organic compounds, resulting in a serious environmental problem. Recent studies have demonstrated a strong positive relationship between biochemical oxygen demand (BOD), nutrient loading of discharged wastewater, and the emergence of eutrophic conditions (Anh *et al.* 2021). Therefore, treatment prior to discharge to meet environmental regulation, which are becoming more stringent, and protect the environment, are required for these purposes. Physical-chemical treatment methods typical have higher infrastructure and operational costs associated than the implementation of a multi-trophic aquaculture (IMTA) approach, which contribute to the reduction of the environmental impacts while adding further income by producing crops with commercial food and/or medicinal uses. However, salinity represents a significant inhibitor to most of the crops, based in glycophyte plants (Duan *et al.* 2022). Even with transgenic or genetic approaches, it has been proven difficult to bread salt tolerant cultivars, due to the complex physiology of salt tolerance mechanisms in plants. So, in this context, the utilization of salt tolerant species (halophytes) is imperative.

Halophytes are able to complete their life cycles at hypersaline (at least 300 NaCl), highly UV radiation and extremely fluctuating temperatures environments, for successive generations (Rodrigues *et al.* 2021). Evolved to live in those stressful conditions allowed these plants to deal with production of free radicals and occurrence of oxidative stress by developing anatomical and biochemical strategies that leads to a production of different metabolites, as phenolic acids vitamins or sterols, many with interest for human use. These adaptations can be a valuable source of biocompounds with antioxidant, anti-inflammatory and antitumor proprieties, that can be used for different commercial proposes. Despite being perfectly adapted to these conditions and all biochemical richness, halophytes only represent about 1% of all plants, and their potential uses are still relative underdeveloped from a meta perspective. Therefore, it is necessary to screen for halophytes with potential to be a valuable asset as feedstock to nutrition, pharmaceutical, ornamental industries and develop their uses (Duan *et al.* 2022).

Based on years of research in halophytes (XtremeBio - CCMAR) and aquaculture (EPPO - IPMA), this line of research, halophytes production with wastewater from marine aquaculture, born to answer the necessity of treat the aquaculture effluents and develop valuable crops with any potential to be used. In our work, we created a system at Pilot Research Station in the southern city of Olhão, Portugal (Fig1), were the wastewater from fish and oyster production is picked up in the decantation tank and led to the greenhouse by pumps and pipes. In the greenhouse, this water is used to cultivate different halophytes that become part of different projects as XtemeAquaCrops and SaltyCrops that already produced a lot of scientific work (Rodrigues *et al.* 2021, Rodrigues *et al.* 2020, Rodrigues *et al.* 2019a and Rodrigues *et al.* 2019b) with different species, sea lavender (*Limonium algarvense* Erben), *Polygnom maritimun* or *Sarcocornia* spp.

Our findings are contributing to the feasibility of contamination-free aquaculture and characterization of halophytes that can be used by different industries.

Figure 1 Overview of the greenhouse implanted inside the pilot research station (EPPO) at Olhão, south of Portugal. (A) Inside view, *Sarcocornia* sp. production; (B) Near view; (C) Aerial view.

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NUTRITIONAL INNOVATIONS IN SUPERIOR GILTHEAD SEABREAM (*Sparus aurata*) GENOTYPES: IMPLICATIONS IN THE UTILIZATION OF EMERGING NEW INGREDIENTS THROUGH THE STUDY OF THE PATTERNS OF SECRETION OF DIGESTIVE ENZYMES

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Introduction

Proficiency can be improved and ecological impact of aquaculture reduced through two key actions a) the use of sustainable ingredients to replace fish meal and fish oil (FM/FO) in the feeds and (b) a successful breeding program addressed to improve growth, feed utilization and fish health. The main objective of the project AQUAIMPACT is to assess possible interactions between fish genotype and nutritive utilization of non-conventional feed ingredients by evaluating a wide range of biological and productive parameters. Among these, total activity and secretion patterns of digestive enzymes offer a highly valuable information on how different factors, linked either to species-specific features or to food composition, may determine the net availability of nutrients. In the present work the aforementioned points were assessed through an experimental design aimed to evaluate a) changes in the levels of activity of different digestive enzymes present in two populations (slow- or fast-growing) seabream (*Sparus aurata*) with different growth trajectories over the production cycle, and b) the effect of different types of protein ingredients on the digestive enzyme profile in both populations of fish.

Materials and methods

Two groups of gilthead sea bream juveniles (288 days after hatching) belonging to two different genotypes (High Growth, HG, from the National Breeding Program PROGENSA® and RF – unselected used as a reference population) were fed for 30 days (3 times a day/6 days a week; 25 fish per tank, 4 tanks/genotype/dietary group) with: (i) a low content of FM/FO control diet based in current commercial formulation (C); (ii) a "future" diet including poultry meal as partial replacer of FM (50%) (F); (iii) a diet using insect meal as partial replacer of FM (33%) (I) and (iv) a diet using single cell proteins from bacterial origin as partial replacer of FM (66%) (CL). Diets F, I and CL replaced totally FO by a combination of poultry oil and DHA oil. Afterwards, fish were used for determination of variations in the pattern of secretion of several digestive enzymes. After receiving the first daily meal, the fish were sequentially sampled at 0, 1, 3, 6 and 9 hours post feeding (5 fish/sample, 45 fish/diet). After separate dissection of stomach and the rest of intestine, crude extracts were obtained by mechanical homogenization of both the tissue and gut contents, followed by centrifugation. Different enzymes (pepsin, trypsin, chymotrypsin, amylase, lipase, leucine aminopeptidase and alkaline phosphatase) were determined using routine protocols. The effect of genotype and diet on total enzyme production, as well as the effect of sampling moment and diet on the time pattern of enzyme production within each genotype, were evaluated by a two-way ANOVA followed by Bonferroni *post-hoc* test when significant differences were found at $p<0.05$. In addition, the differences in values of each enzyme activity obtained at different sampling moments for a given diet were evaluated by one-way ANOVA followed by Bonferroni *post-hoc* test.

Results

Significantly higher ($p<0.05$) values of pepsin and chymotrypsin were obtained in HG fish compared to RF. The effect of the diet was only significant in the case of aminopeptidase, with higher ($p<0.05$) values measured in fish fed the CL diet when compared to fish fed the rest of the dietary treatments and irrespective of the fish genotype. In contrast, the effect of diet was evidenced when the two genotypes were evaluated separately and showed a significant effect on the secretion of pepsin, amylase and aminopeptidase in fish belonging to the HG group, but no effect on those of the RF group. The effect of time after ingestion of a meal was significant ($p<0.05$) for all the enzymes in the HG group, with the exception of lipase and phosphatase and similarly significantly influenced the activity of all the enzymes, with the exception of pepsin, in the RF group.

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Time secretion patterns of enzymes in fish fed on different diets were more evident in the HG group, with significant differences ($p<0.05$) for pepsin, amylase and aminopeptidase. In contrast, with the exception of amylase, no significant differences related to diet type were observed in the pattern of enzyme secretion within RF group. Three types of patterns were identified in response to feeding classified as: (a) *Quick response*; characterized by a sharp increase of values measured 1 h after meal supply followed by a steady decrease and maintenance of values for the rest of sampling time; (b) *Delayed response;* characterized by greater variations and peaks observed from 3 to 6 hours after feed intake and (c) *No variation;* characterized by the absence of variations in enzyme activity as a response to meal supply.

HG fish presented a quick response for pepsin in fish fed on diets CL, F and I compared to fish fed C diet, which did not present variations in the activity of pepsin during the experiment. In the case of RF fish, the quick response for pepsin activity was detected in fish fed CL, F and C, whereas fish fed I diet did not present variations. HG fish presented a delayed response for trypsin, chymotrypsin, alkaline phosphatase and to a lesser extent in amylase and aminopeptidase, being more evident in fish fed CL and I diet than in fish fed C and F diets. In RF fish trypsin, chymotrypsin and amylase presented a delayed response pattern, however the maximum levels of enzyme production were generally observed much earlier that in the HG group, around 3 h post feeding. HG fish presented almost no variation in lipase activity along the response pattern, and for RF fish presented a similar pattern for lipase, aminopeptidase and alkaline phosphatase activities.

As a resume, the above detailed results suggest the existence of significant differences between both fish genotypes in terms of total production of several digestive enzymes as well as in the patterns of their secretion after feeding. In addition, the composition of the diets also influenced both parameters; higher enzyme production was observed in fish of the HG group receiving diet CL, but lower in those fed on diets F and C, and a similar response was observed in fish in the RF group. These results may partly explain the differences in growth and feed efficiency observed between both genotypes when fed on the different diets.

ASSESSING THE IMPACT OF DIFFERENT EXPERIMENTAL DIETS ON BALLAN WRASSE (*Labrus bergylta*) LARVAE GROWTH PERFORMANCE

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Introduction

Ballan Wrasse became an important eco-friendly alternative for the biological control of sea lice on farmed Atlantic salmon (Kvenseth 1996 and Skiftesvik *et al*. 2013). Despite the improvements on hatchery production over the last years, there are still some bottlenecks, especially at the early larval development stages. In particular, the weaning stage can be characterized by low survival and growth rates, and incidence of deformities (Kousoulaki *et al*. 2015). The aim of this study is to assess the effect of various diets with different protein sources on the growth performance of ballan wrasse larvae.

Material and methods

The Ballan wrasse (*Labrus bergylta)* larvae trial was performed at Otter Ferry Seafish facilities following the commercial husbandry and feeding protocols. It lasted for 44 days, from 31-75 Days After Hatch (DAH), at a temperature of 12 ± 0.8 °C. Larvae had an initial weight of 0.001±0.1g. Four diets were tested in quadruplicate: two commercial controls (1 and 2) and two experimental diets (A and B) with different sources of protein, both produced at SPAROS. Experimental diet A (60% of crude protein) had krill, squid and shrimp meals as main protein sources whereas diet B (58% of crude protein) had krill and squid meals as main protein sources. Both diets had 15% of crude fat with similar levels of n-3 HUFAs.

Results

Ballan wrasse larvae fed diet B showed significantly higher Dry Weight (DW) at 50 DAH ($P<0.05$) (Fig.1). At 75 DAH, Total Length (TL) was significantly higher in fish fed diet B ($P<0.05$).

Discussion and Conclusion

Different dietary protein sources had an impact on Ballan wrasse larvae growth. It was observed that larvae fed with a mixture of krill and squid meals as main protein sources (Experimental diet B) showed a tendency for higher growth performance when compared to fish fed the other diets. Overall, results support the fact that the use of different marine raw materials in microfeeds for Ballan wrasse larvae may influence weaning success (Kousoulaki et al. 2015). It also supports the need of tailored microfeeds for different fish larval species (Navarro-Guillén et al. 2019).

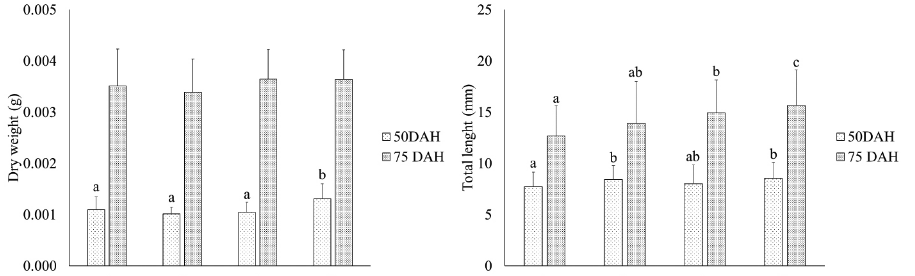

Figure 1.
Dry Weight (DW) and Total Length (TL) of Ballan wrasse larvae fed commercial diets (1 and 2) and experimental diets A and B at: 50 DAH, n=3 for DW and n=25 for TL; and 75 DAH, *n*=3 for DW and n=30 for TL. Results are expressed as means ± standard deviation. Different lowercase letters indicate significant differences between the dietary treatments ($P<0.05$).

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TASTE BEYOND ORAL PERCEPTION IN SEABREAM *Sparus aurata*: NEW EVIDENCE FOR FUNCTIONAL GUT CHEMICAL SENSING IN FISH

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Introduction

Taste receptors (TRs) are classically associated to the perception of gustatory sensations in oral tissues. However, many TRs are also found in different body tissues including the gastrointestinal tract (GIT), where they perform important roles (Finger and Kinnamon, 2011). In the GIT, the presence of TRs in specialized cells (enteroendocrine cells: EECs) regulates digestive, absorptive and metabolic functions through gut sensing mechanisms (Alpers, 2010). Functional actions of TRs are associated to their co-expression with gut peptides, including ghrelin (GHR), cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) or peptide YY (PYY), to cite a few. Once a TR is activated, an intracellular signaling cascade is initiated, terminating with peptide secretion in the EEC basolateral side, which can signal neighboring (paracrine action) or remote (endocrine action) cells, or activate afferent neurons (neural action). The taste receptor type 1 (T1R) family is one of the TR types that is commonly expressed in EECs, acting as heterodimeric sensors of nutrients in the mammalian gut: T1R1/T1R3 responding to umami compounds such as amino acids, and T1R2/T1R3 to sweet substances. We previously showed the expression of the *Sparus aurata* (*sa*) T1R gene repertoire in oropharyngeal, GIT and brain tissues. However, this does not necessarily imply a conserved role in gut sensing. Furthermore, expression in GIT was observed in early larval stages but no expression was found in oropharyngeal tissues up to 12 days post-hatching (dph), which was puzzling.

The present study aimed to provide direct evidence for mRNAs co-expression of *sa*T1R genes (mostly *t1r3*, as the common element of both receptors) with EEC-type peptides such as *ghr*, *cck*, *pyy* and proglucagon (*pg*), to establish a morphological link indicating possible roles of T1R in gut nutrient-sensing mechanisms and in the regulation of fish digestive processes. A second objective was to extend the period of investigation during larval ontogeny to establish the temporal pattern of expression in GIT and oral tissues for the entire *sa*T1R gene repertoire in relation to first-feeding.

Materials and methods

Whole-mount in situ hybridization (WISH) techniques were used to localize *sa*T1Rs mRNA expression in whole larvae in five selected stages, including yolk-sac nourishment (5 dph) and exogenously feeding (on rotifers) larvae at four stages (11, 14, 17 and 21 dph), up to the appearance of a mature digestive system in this species.

Chromogenic in situ hybridization (CISH) and dual fluorescent ISH (FISH) methods were used to localize and co-localize the mRNA expression of selected *sa*T1Rs (*t1r3*, *t1r1* and *t1r2b*), taste signaling molecules (*giα1* and *giα2*), gut peptides (*ghr*, *cck*, *pyy* and *pg*), and a specific marker of undifferentiated and mature EECs (neurod1: *nd1*), in the different areas of the GIT from stomach to posterior intestine, in adult seabream.

Results and discussion

In pre-feeding larvae, mRNAs for the seven *sa*T1Rs were found in the stomach and foregut, and expression in posterior intestine was also observed for *t1r2b*. At 11 dph, expression patterns remained essentially the same in the developing GIT, and even though exogenous feeding had already started, *sa*T1R expression was not detected in oral taste tissues (lips, tongue, oral cavity epithelium). During post-feeding stages, mRNAs became gradually detected in the oral cavity epithelium and/or tongue and pharynx, while overall maintaining a strong expression in the stomach and intestine.

In adult fish tissues, presumptive EECs were firstly identified in different positions of the GIT mucosa using *nd1* as a specific marker. Then, it was demonstrated that *sa*T1R genes are mostly expressed in cells that are consistent with mature EECs, co-localizing with taste signaling molecules (*giα1* and *giα2*), and that heterodimerization is a conserved mode of TR coupling in fish tissues, although single expression is also found (as in mammals). Finally, some cases of co-localization between *t1r3* and *ghr*, *cck*, *pyy* and *pg* genes in different regions of the GIT were identified, as well as independent and non-overlapping expression domains, as described also in mammals.

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Table 1. Summary of gut *vs* oral sites of *sa*T1R(s) expression throughout ontogenesis.

T1R(s)	5 dph	11 dph	14 dph	17 dph	21 dph
t1r1	Gut	Gut	Gut	Gut/Oral	Gut/Oral
t1r3	Gut	Gut	Gut	Gut/Oral	Gut/Oral
t1r2a	Gut	Gut	Gut	Gut	Gut/Oral
t1r2b	Gut	Gut	Gut/Oral	Gut/Oral	Gut/Oral
t1r2d	Gut	Gut	Gut	Gut/Oral	Gut/Oral
t1r2e	Gut	Gut	Gut	Gut	Gut/Oral
t1r2f	Gut	Gut	Gut	Gut	Gut

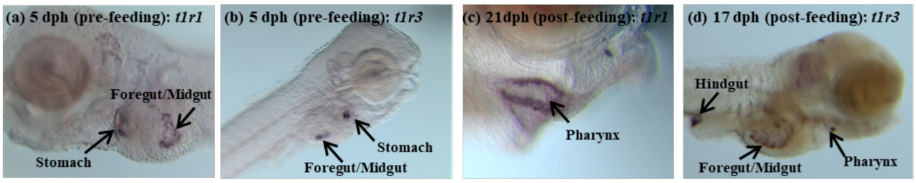

Fig. 1. Exemplificative WISH images obtained for larval *t1r1* (a, c) and *t1r3* (b, d) expression prior to (a, b) or after (c, d) the start of exogenous feeding.

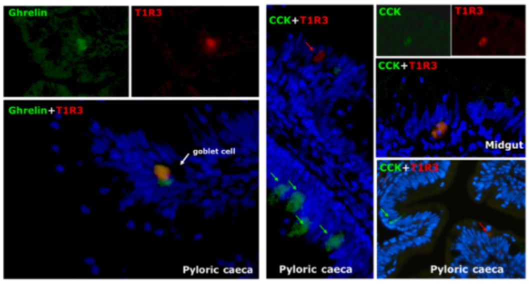

Fig. 2. Exemplificative dual FISH images from adult tissues showing both single and co-expression of T1R (*t1r3*) and gut peptides (*ghr*, *cck*) mRNAs in pyloric caeca.

In conclusion, the co-localization of *sa*T1Rs with neuroendocrine gut peptides has been clearly documented in cells with EEC-like morphology, along the GIT. This provides important evidence supporting their plausible implication in the modulation of gut hormone secretion, which had not been clearly established so far in a fish species. Furthermore, the confirmed expression of all *sa*T1R genes in the larval gut before first feeding, even prior to oral tissue expression, suggests their involvement in important GIT physiological processes, and that the onset of gene expression is not induced by food but rather genetically programmed.

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PALATABILITY ENHANCEMENT OF RAINBOUT TROUT AND ATLANTIC SALMON DIETS

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Introduction

Maintaining a good feed intake is crucial for aquaculture productivity, to guarantee an adequate intake of energy and nutrients in growing animals. However, fish feeding can be affected by many factors, including poor palatability of dietary ingredients (failing to provide attractive sensory cues), changes in the diet to which the animal is accustomed, or other factors that affect the animal's motivation to feed, including stress, poor health or sub-optimal environmental conditions. Fish species, and salmonids in particular, have highly sensitive olfaction and gustation. These sensory systems are key to detect and identify food items (mainly olfaction) and promote selective food intake (mainly gustation). Therefore, by modifying the organoleptic qualities and enhancing attractive chemical cues of aquaculture feeds, it is possible to optimize feed intake in farmed fish.

In the present study, an experimental protocol developed for testing the palatability of feeds and feed intake under dietary changing conditions (i.e., with a challenge of diet "novelty") was used to assess the efficacy of palatability enhancers (PE) designed to improve feed intake of low palatability diets for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*).

Materials and methods

Two trials were performed in 60 L cylindroconical tanks equipped with an INRA automated feces collection system, originally developed for digestibility studies but adapted as an experimental tool to quantify feed intake. The tanks were operated as an open-flow freshwater system in which the tank outlet water is sieved by moving grids, and all suspended residues are separated from the water and projected into a metallic tray. This allows a full recovery of all uneaten pellets (Figure 1). Trial 1 was performed with 20 rainbow trout per tank, with mean body weight (BW) of 74 ± 6 g and at an average temperature of 14.3ºC. Trial 2 was done with 20 Atlantic salmon per tank with BW of 18 ± 1.7 g and at an average temperature of 12.6ºC.

Each trial tested a positive control (PC) diet of high palatability (with very high contents of marine ingredients; Table 1), a negative control (NC) diet of reduced palatability (low level of marine ingredients and high in plant protein and oil sources; Table 1), and experimental diets with the same basal formulation as the NC but supplemented with various test additives at 0.1%. All test additives form stable oil suspensions and were blended in oils and applied post-extrusion by coating. Each tank was fed on a diet during one week and each week a new experimental feed was introduced, until each diet had been tested in 4 different tanks (i.e., without the repetition of a given diet in the same tank). Therefore, a total of 4 replicates (tanks) were used per treatment, each replicate representing the average feed intake during one week.

Fish were fed twice a day (at 10.00 and 15.00h), in excess of the maximum feed intake estimated by feeding all tanks on the PC diet for one week before starting the trial (WK0). Every day, during 20 minutes after each meal, the uneaten feed pellets from each tank were recovered, dried overnight in a convention oven, and quantified in a dry basis. Feed intake was calculated as a % of fish biomass, by subtracting the uneaten dry feed from the distributed dry feed.

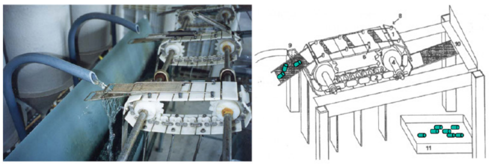

Fig. 1. INRA feces collection system used for the collection of uneaten pellets.

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Table 1. Main ingredients (>2%) in experimental basal formulations.

	Trial 1 (Trout)		Trial 2 (Salmon)	
Ingredients (% of feed)	PC	NC	PC	NC
Fishmeal LT70	22.75	12.75	30.00	7.50
Fish protein hydrolysate	5.50		5.00	
Squid meal	5.50			
Krill meal	15.00		5.50	
Soy protein concentrate		17.50	9.00	20.50
Pea protein concentrate				15.00
Wheat gluten		7.65	7.00	15.00
Corn gluten meal		8.00	5.00	5.00
Soybean meal 48		10.00		
Wheat meal	28.15	15.50	15.50	10.10
Faba beans (low tannins)	5.00	5.00	5.00	5.00
Fish oil	16.90	1.20	16.80	4.00
Soybean oil		9.50		
Rapeseed oil		9.50		14.40

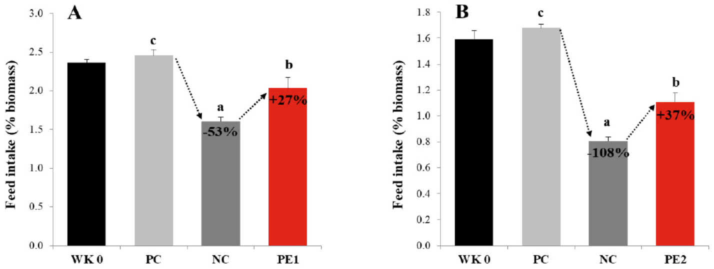

Fig. 1. Feed intake (% biomass) in trial 1 (A) and trial 2 (B). PE1 and PE2 are different palatability enhancer additives. Columns are means of n=4 tanks (1 week duration) ± standard deviation. Letters indicate P<0.05 (one-way ANOVA).

Results and discussion

The palatability trials enabled screening different PE additives and identifying those with the highest phagostimulant effect in both salmonid species (Figure 2, showing the most effective test additive in each trial). Results show that the intake of the PC diet remained high and constant during the duration of the trials, and similar to the maximal level assessed for all tanks on the pre-trial week (WK0). Feed intake was highly and significantly reduced on the NC treatment, compared to the PC – 53.3% less in rainbow trout and 107.9% less in Atlantic salmon. However, the palatability of the NC diet could be significantly improved by supplementing with 0.1% of PE1 in rainbow trout (+26.9% feed intake) or with 0.1% of PE2 in Atlantic salmon (+37.2% feed intake).

In conclusion, the results confirm that the experimental protocol enables a precise measurement of feed intake in juvenile fish, presenting a good methodology to identify palatability enhancers for fish feeds. Furthermore, it is noteworthy that despite being closely related, the most effective PE differed among the two species, supporting that fish have remarkable species-specificity in taste preferences (Morais, 2017).

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MODULATION OF PHYSIOLOGICAL RESPONSES IN ATLANTIC SALMON EXPOSED TO STRESSOR CONDITIONS: HYDROLYZED *Debaryomyces hansenii* IN FUNCTIONAL FEEDS CAN IMPROVE FISH HEALTH AND WELFARE

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Introduction

The salmon farming industry faces biological challenges related to multi-stressor conditions (e.g., physical, infectious and environmental problems) that impact fish health and welfare, and cause economic losses (1). To address these problems, the use of microbial ingredients (MI) in functional feeds to improve overall fish health is gaining interest (2). Yeast as MI contain a wide range of Microbe-Associated Molecular Patterns (MAMPs) such as β-glucans, α-mannans and nucleic acids, which act on host pattern-recognition receptors (e.g., Toll-like receptors and C-type lectin receptors). This can induce both local and systemic physiological effects by modulating cytokines and effector molecules that also regulates the stress response, as well as coordinating the antigen presentation process (1, 3-5).

A promising MI for aquafeeds is *Debaryomyces hansenii*. It has already been described in different vertebrates that selected inactivated fractions (e.g., cell wall components and polyamines) from this non-Saccharomyces yeast species have been able to modulate the expression of immune-related biomarkers (NFϰB, IL-1β, and TNFα) and to increase phagocytic ability and the production of reactive nitrogen species in leukocytes (6, 7).

In our study, we have focused on the use of two distinct hydrolyzed *D. hansenii*-based products (LAN4 and LAN6 from Lallemand SAS), to evaluate their effects on immune-related biomarkers of Atlantic salmon exposure to short-term hypoxia stress or to stress associated with seawater transfer. This knowledge will facilitate the inclusion of bioactive components based on *D. hansenii* in functional feeds for salmonids, with the potential to modulate physiological responses in fish exposed to challenging conditions.

Materials and Methods

A fish trial was conducted for 13 weeks (7 weeks in freshwater and 6 weeks in seawater) with vaccinated Atlantic salmon fed a commercial-like control diet (CD) or CD containing 0.1% LAN4 (marine origin) or 0.1% LAN6 (dairy origin) in triplicate (Figure 1). At the end of freshwater phase, fish from each dietary group were transferred to seawater, while those that remained in freshwater were subjected to an acute stress challenge (1 min hypoxia; 8). For hypoxia stress test, plasma, gills and distal intestine samples were collected before stress (pre-stress), immediately after (0-h post-stress), 1, 3, 6, 12 and 24-h post stress. Regarding the fish transferred to seawater, a natural outbreak of *Moritella viscosa* was detected after 5 weeks (week 12) and a week later (week 13), plasma, gills, distal intestine and liver samples were taken from all fish. We combined transcriptomic strategies with detection of specific phenotypic biomarkers by carrying out RNAseq, ELISA and morphometric analysis in the samples.

Results

Compared to the control diet (Figure 1) and following acute hypoxia, fish fed LAN4 were able to prevent the secretion of plasma cortisol and IL-10, which are both biomarkers associated with immunosuppression. Moreover, gills showed differentiated gene expression of metabolic pathways associated with stress tolerance and oxidative regulation, and in the intestine, goblet cells maintained higher levels of mucins, that are proteins involved in the protection of the intestinal tract. Interestingly, while plasma total IgM levels after the *M. viscosa* outbreak in seawater showed no difference between dietary groups, there was (in fish fed LAN6) a 14% increase of specific IgM against *M. viscosa*, in addition to an upregulation of genes related to humoral immune response and complement activation in liver.

Conclusions and perspectives

Functional feeds containing a specific strains of hydrolyzed *D. hansenii* yeast have the potential to differentially modulate health parameters and stress response, hence can contribute to improve welfare in intensive aquaculture. We are thus currently evaluating the ability of *D. hansenii* yeast to induce trained immunity and support the capacity to withstand multi-stressor conditions in order to build-up a more resilient Atlantic salmon.

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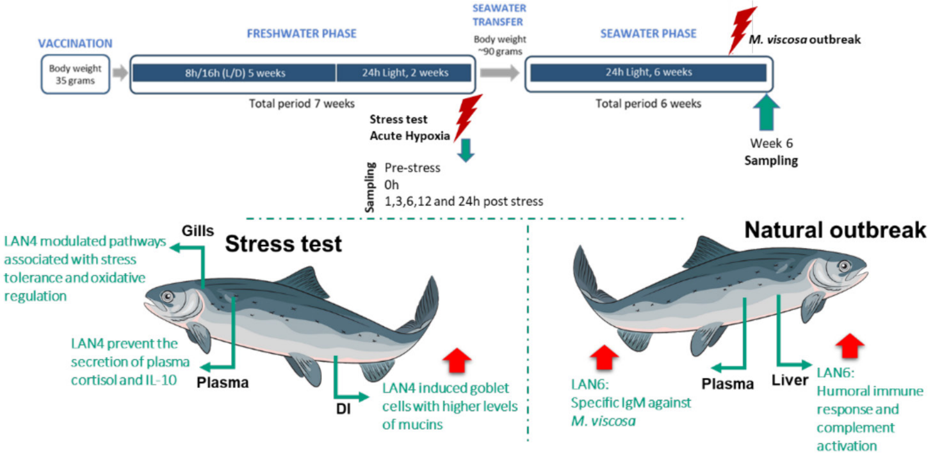

Figure 1. Experimental design and results.

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USING WIRELESS METHODS TO REMOTELY ASSESS STRESS IN FARMED ATLANTIC SALMON *(Salmo salar)*

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Introduction

Aquaculture is one the fastest growing food sectors worldwide requiring increased levels of industrialization and intensification. A vital aspect of sustainable aquaculture is to safeguard the welfare of the animals, a practice benefiting not just the farmed fish, but also the farmers and consumers, as it improves the quality and cost-efficiency of the product. The recent development, miniaturization and greater accessibility to biosensing technologies (*e.g*., data storage tags) have allowed for their increasingly widespread adoption in animal research, which may pave the way for their routine implementation in aquaculture settings to monitor for early warning indicators of poor welfare (Brijs et al., 2021; Føre et al., 2018). Still, to test the validity of these sensor data as welfare indicators, further analyses establishing correlations between the sensor values and blood parameters are required. Towards this goal, we investigated how well parameters measured using data storage tags such as heart rate and swimming activity at different conditions (*i.e*., rest, elevated swimming speeds, stress and recovery) correlate with blood parameters often used as indicators of acute stress and poor welfare.

Materials and methods

Atlantic salmon (*Salmo salar*, n = 13, body mass = 1207.2 ± 65.1 g) were simultaneously implanted with an intravascular venous catheter for blood sampling and a data storage tag capable of measuring ECG and activity (via 3D accelerometer). The fish were then transferred to a swim respirometer, where oxygen consumption was measured overnight at a swimming speed of 0.5 body lengths s^{-1} (BL s^{-1}). After the overnight recovery period, swimming speed was increased in 0.5 BL s^{-1} steps to 1.5 BL s^{-1}, following which the fish were netted out of the respirometer, thus eliciting stress, and transported to a holding tank, where they were allowed to recover for 24 hours. Throughout the protocol, blood samples were obtained *via* the catheter at six timepoints: at rest (0.5 BL s^{-1}), 1.0 BL s^{-1}, 1.5 BL s^{-1}, immediately post stress, 1 hour post-stress and 24 hours post-stress. Blood parameters measured include cortisol levels, venous oxygen partial pressure, pH, lactate levels, osmolality, hematocrit, hemoglobin concentration, blood ion composition and glucose concentration. Additionally, we videorecorded the fish during the swim trial, allowing us to measure ventilation rate and tail beat frequency.

Results and discussion

Preliminary results are provided in Figure 1, although further blood analyses are currently being performed. Heart rate, activity and oxygen consumption rate increased with swimming velocity, while partial pressure of oxygen and pH decreased. pH was lowest following stress, although it was restored to resting levels 1 hour post-stress. Lactate concentration increased at 1.5 BL s^{-1}, indicating increase in anaerobic metabolism, and peaked 1 hour post-stress. Pearson correlation analysis of the available data showed that heart rate was significantly positively correlated to activity and, both heart rate and activity were correlated to oxygen consumption rate. Heart rate was negatively correlated to lactate concentration, while activity was negatively correlated to pH and venous partial pressure of O_2. In conclusion, these preliminary results indicate that heart rate and activity correlate with oxygen consumption rate and other stress-driven cascades of physiological events that can be measured in the blood, providing further evidence that biosensors can be used as a tool to estimate stress in aquaculture. This can be used to facilitate the correction of harmful husbandry practices that lead to exacerbated stress, thus reducing the likelihood of long-term negative consequences to the welfare of the fish.

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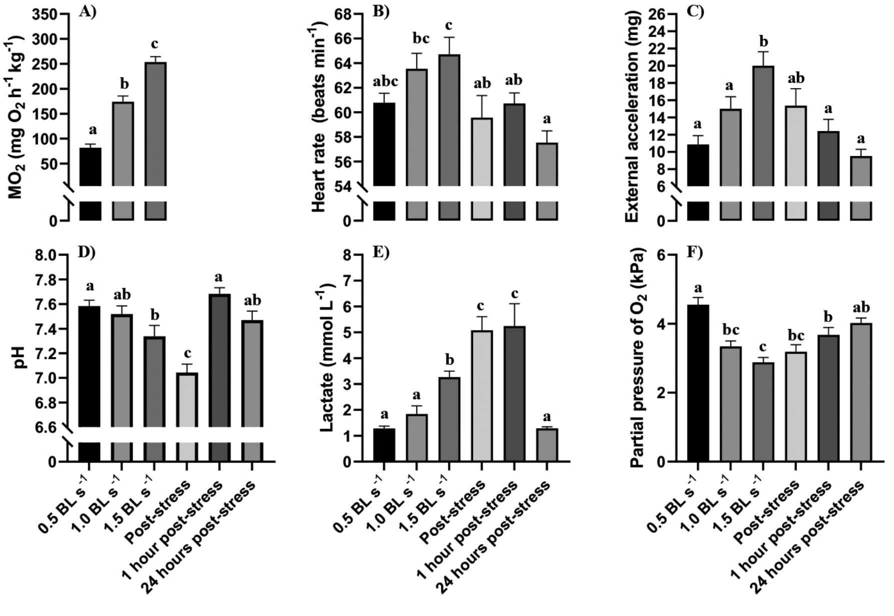

Figure 1. A) Oxygen consumption rate (MO_2), B) heart rate, C) external acceleration (activity), D) pH, E) lactate and F) partial pressure of O_2 at different conditions. Different letters indicate significant differences. Data are means ± SEM.

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SCALING KELP FARMING AS A NATURE-BASED SOLUTION

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Research continues to bolster widely recognized ecosystem services of kelp farming activities, including uptake of excess nutrients, mitigation of ocean acidification, and habitat creation. Not only do farms contribute to carbon sequestration, but the harvested biomass can be used in value chains that offset global resource use. Kelp farms also improve coastal community resilience through new economic opportunity. Their benefit to both people and nature make kelp farms an important nature-based solution in many regions.

In order to unlock the potential for kelp farming as a nature-based solution, barriers to growth must be addressed. WWF-US is two years into implementation of a strategy reducing barriers for the responsible expansion of the seaweed industry in underdeveloped seaweed farming regions, namely the North Atlantic and Eastern Pacific. Barriers include access to capital for farmers and companies, developing market demand, and achieving social license to operate more farms in coastal areas. Environmental NGOs recognizing the contribution of kelp farming to effectively restore ecosystems and address societal challenges as a nature-based solution have a key role to play in improving public buy-in for the industry, allowing production to scale up.

This presentation will explore how, in conversation with local communities, there is room for farms to increase, a necessary step to achieving meaningful impacts on climate and ecosystems, and function as a nature-based solution.

THE EFFECT OF POLYPROPYLENE MICROPLASTICS AND CHEMICAL POLLUTANTS ON EUROPEAN SEA BASS (*Dicentrarchus labrax*) GUT MICROBIOTA

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Introduction

It is generally accepted that plastics, consisting mainly of micro-sized (1 µm–1 mm) and nano-sized (<1 µm) particles, have become a serious threat to marine ecosystems, not only because they can physically damage the organisms, but also due to the toxicity of the associated chemical pollutants (Jambeck et al., 2015). Microplastics (MPs), which are consumed by marine organisms that confuse them with food sources, can causes various adverse effects. In fish, environmental chemicals can effectively induce changes in gut microbiota. Therefore, a dysbiosis might develop in the host's intestine after consuming MPs due to the ingestion of foreign and potentially pathogenic bacteria or to chemicals that make up or adhere to MPs (Fackelmann and Sommer, 2019). The consequences of exposure to MPs and the combined effects of MPs and adsorbed pollutants, on the microbiota community has not been exhaustively investigated. Accordingly, we exposed European sea bass juveniles to chemical pollutants and to MPs contaminated or not with a blend of chemical pollutants (dichlorodiphenyldichloroethylene (p,p'-DDE), chlorpyrifos (CPF), and benzophenone-3 (BP-3)), by adding them to the fish diet. The aim of the study was to determine the individual and combined effects of dietary MPs and chemical pollutants on the fish gut microbiota population.

Materials and Methods

The feeding trial was set in a flow-through marine water system with three hundred European sea bass (80.91 ± 13.28 g), randomly distributed in twelve tanks (500 l). Four different diets were formulated. Commercial diet with no additives was considered as the control diet, whereas diet MP, diet P and diet P+MP were manufactured adding to the commercial diet: MPs alone, or a mix of three chemical pollutants, or MPs contaminated by the same mix of chemical pollutants, respectively. The level of inclusion of MPs in the diets was 10% (w/w), while, for diets P and P+MP the blend of the three chemical pollutants was composed by p,p'-DDE, CPF, and BP-3 in concentration of 1000, 100, and 300 ng/g, respectively. At the end of the trial, two fish per replicate tank (6 fish/diet) were sacrificed to collect the fecal matter and the autochthonous intestinal bacteria for gut microbiome analysis. The sequencing was carried out using Illumina MiSeq platform and processed using QIIME™ 2 (v. 2018.4) then, the representative sequences from each operational taxonomic units (OTUs) were aligned to Greengenes database v. 13.8 down to genus taxonomical level.

Results and Discussion

In line with previous studies (Wan et al., 2019), the present metagenomic analysis showed an alteration in sea bass gut microbiome composition and in bacterial species richness following the ingestion of pollutants or polluted MPs. Furthermore, the PCoA analysis revealed that pollutant treatments significantly altered gut microbiome community structures that clearly separated them from the control group. Irrespective of the diet, the gut microbiota of European sea bass was dominated by Firmicutes, Proteobacteria, and Actinobacteria phyla, as already reported in previous microbiome studies in this species (Rimoldi et al., 2020). However, their relative abundances were significantly changed by ingesting pollutants. In particular, compared to the control group, although the dietary inclusion of virgin MPs did not cause relevant changes in microbiome, the polluted diets increased the abundance of the phylum Proteobacteria, widely considered a microbial signature of gut inflammation (Shin et al., 2015), while reduced the Firmicutes content, which instead are generally considered beneficial and associated with a healthy intestine. These bacteria in fact, are known for having anti-inflammatory potential and a crucial role in regulating the host immune system (Terova et al., 2016). These results are in line with those reported for yellow croaker (*Larimichthys crocea*) and adult zebrafish (*Danio rerio*) exposed to PS-MPs (Jin et al., 2018). In agreement with the reduction in beneficial bacterial taxa belonging to Fimicutes phylum, PICRUSt analysis predicted a decrease in starch and carbohydrate metabolism as well as in cysteine, methionine, and purine metabolism. On the contrary the two-component and secretion system pathways were instead greatly upregulated. This would agree with the gut inflammatory signs and microbiome profiles observed in fish fed diets containing chemical pollutants. Likewise, an increase in lipopolysaccharide biosynthesis could be related to the inflammatory status of intestine and to dysbiosis.

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Conclusion

In summary, our results clearly demonstrate a significant perturbation in gut microbial communities, including the reduction in beneficial bacteria genera and the increase in potentially pathogenic microorganisms. The results obtained in this study provide, therefore, new insights into the potential risks of ingesting MPs as pollutant carriers in marine fish.

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BLACK SOLDIER FLY OIL IN DIETS FOR GILTHEAD SEABREAM (*Sparus Aurata*) JUVENILES: EFFECTS ON DIGESTIBILITY AND DIGESTIVE ENZYMES ACTIVITY

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Introduction

In recent years, insects have emerged as a more sustainable alternative ingredient for aquafeeds. Although the potential of insect meals as protein replacement has already been established, very limited information is available regarding the inclusion of insect lipids. Black soldier fly (*Hermetia illucens*) larvae, one of the most promising insect species, are rich in fat (25-35%), with a fatty acid profile mainly composed of medium-chain fatty acids (MCFA), mainly lauric acid (C12:0) [1,2] These MCFA are rapidly catabolized and preferably used as an energy source rather than deposition as an energy reserve, thus making insect oil an interesting ingredient for use in aquafeeds. Therefore, this study aimed to evaluate the inclusion of *Hermetia illucens* larvae oil (HIO) as a replacement for vegetable oils (VO) in diets for gilthead seabream (*Sparus aurata*) juveniles.

Materials and methods

A control diet (HIO0) was formulated to include 3% fish oil and 10% VO mix (50% linseed, 30% palm, and 20% rapeseed oils). Three other diets were formulated to include HIO at increasing levels (4, 8, and 10%), replacing 33, 66, and 100% of the VO mix (diets HIO33, HIO66, and HIO100, respectively). All diets were tested in triplicate in a growth trial with gilthead seabream juveniles (60g) hand-fed to apparent visual satiation for 10 weeks. At the end of the trial, the fish intestine was collected to assess the dietary effects on the activity of digestive enzymes. A digestibility trial was also conducted to determine the apparent digestibility coefficients (ADC) of the diets.

Table 1: Apparent digestibility coefficients (ADCs) of gilthead seabream juveniles fed the experimental diets.

							Polynomial contrasts	
	HIO0	HIO33	HIO66	HIO100	SEM	ANOVA P-value	Linear P-value	Quadratic P-value
Dry matter	53.3	50.5	48.5	57.7	2.37	0.238	0.118	0.155
Protein	90.1	88.7	88.7	91.2	0.52	0.068	**0.046**	0.056
Lipids	85.3	84.7	82.9	86.7	0.55	0.068	0.064	0.038
Energy	70.1^{b}	67.3^{b}	72.0^{ab}	76.2^{a}	1.37	**0.004**	**0.002**	0.079

Means of n=4. SEM: standard error of the mean. Different superscript letters indicate significant differences between treatments (one-way ANOVA, p<0.05).

Table 2: Activity of digestive enzymes of gilthead seabream juveniles fed the experimental diets.

							Polynomial contrasts	
	HIO0	HIO33	HIO66	HIO100	SEM	ANOVA P-value	Linear P-value	Quadratic P-value
Trypsin	77.9	83.0	79.8	72.7	4.38	0.877	0.647	0.505
Total Proteases	1.03	1.25	1.27	1.04	0.05	0.159	0.925	**0.025**
Lipase	3.55	3.29	3.13	3.21	0.13	0.732	0.345	0.531
Amylase	206.5	159.1	158.1	131.4	10.3	0.181	**0.038**	0.788

Trypsin, Lipase, and amylase activity: nmol mg protein^{-1}; Total proteases activity: nmol mg protein^{-1} min^{-1}. Values are presented as a mean of n=9. SEM: standard error of the mean. The absence of superscript letters indicates no significant differences between treatments (one-way ANOVA, p> 0.05).

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Results and Conclusion

Results indicate that the dietary inclusion of HIO increased the diet's energy digestibility (Table 1). The ADC of dry matter and lipid were not affected, while the ADC of protein showed a positive linear trend. While no significant effects were observed in the digestive enzyme activity (Table 2), a decreasing trend was observed in amylase activity.

Overall, these results suggest that gilthead seabream juveniles can effectively utilize *Hermetia illucens* larvae oil without detrimental effects on nutrient digestibility and activity of digestive enzymes. Further studies are required to understand the impact of HIO on lipid metabolism, oxidative stress response, and immune status of gilthead seabream and also the implications on fillet quality traits.

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EFFECTS OF DIETARY INCLUSION OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE OIL ON LIVER AND INTESTINAL OXIDATIVE STRESS OF GILTHEAD SEABREAM JUVENILES

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Introduction

Black soldier fly (*Hermetia illucens*) larvae have already been established as one of the most promising insect species to be included in aquafeeds. They have a high protein content, a balanced amino acid profile, and are also able to convert low-quality waste materials into high-valuable ingredients. Most of the research has been focused on its protein portion and so, limited information is available on the use of *H. illucens* larvae oil (HIO) in fish feeds. *H. illucens* larvae are rich in lipids (25-35%), mostly composed of medium-chain fatty acids (MCFA), namely lauric acid (C12:0), with great potential to be used as an energy source (1) and have also been reported to exhibit antioxidant, antimicrobial, and immune-modulating properties in animals (2,3). Therefore, this study aimed to evaluate the effects of the dietary inclusion of *H. illucens* larvae oil (HIO) on the hepatic and intestinal antioxidant response of gilthead seabream (*Sparus aurata*) juveniles.

Materials and methods

Four experimental diets (3% fish oil) were formulated to replace a vegetable oil mix (containing 50% linseed, 30% palm, and 20% rapeseed oils) with HIO at increasing levels: 0, 33, 66, and 100%, corresponding an inclusion level of 0, 4, 8, and 10% (diets HIO0, HIO33, HIO66, and HIO100, respectively). These diets were tested in triplicate groups of gilthead seabream juveniles that were hand-fed to apparent visual satiation for 10 weeks. At the end of the trial, the liver and intestine of 9 fish per dietary treatment (3 fish/tank) were collected to assess the effects on lipid peroxidation and the activity of selected antioxidant enzymes: glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), and catalase.

Table 1: Lipid peroxidation and activity of selected antioxidant enzymes of gilthead seabream fed the experimental diets

							Polynomial contrasts	
	HIO0	HIO33	HIO66	HIO100	SEM	ANOVA P-value	Linear P-value	Quadratic P-value
Liver								
LPO[1]	6.41	4.16	6.26	5.23	0.39	0.134	0.663	0.367
GR[2]	5.0^{b}	5.87^{ab}	5.34^{ab}	6.38^{a}	0.17	**0.019**	**0.014**	0.783
G6PDH[3]	70.4^{b}	84.6^{ab}	83.0^{ab}	103.5^{a}	4.26	**0.039**	**0.008**	0.688
CAT[4]	248.7	252.9	241.8	316.5	10.7	**0.040**	**0.036**	0.082
Intestine								
LPO[1]	9.36^{a}	5.10^{b}	4.72^{b}	5.44^{b}	0.48	**0.001**	**0.002**	**0.003**
GR[2]	3.90	4.00	4.20	3.67	0.11	0.427	0.632	0.165
G6PDH[3]	2.17	2.48	2.58	2.86	0.14	0.369	0.085	0.948

[1]LPO – Lipid peroxidation (MDA/g tissue); [2]GR - glutathione reductase (mU/mg protein): [3]G6PDH: glucose-6-phosphate dehydrogenase (mU/mg protein); [4]CAT: catalase (mU/mg protein).
SEM: Standard error of the mean

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Results and conclusion

Present results indicate that the dietary inclusion of HIO had no effects on the activity of intestinal antioxidant enzymes, while lipid peroxidation was significantly reduced when compared to the control diet. Liver lipid peroxidation was also not affected by the experimental diets, but an increase in GR and G6PDH activity was observed, being the highest at the highest inclusion level of HIO. Catalase activity also linearly increased with the inclusion of HIO.

These findings suggest that *H. illucens* larvae oil can reduce intestinal lipid peroxidation while increasing the antioxidant response in the liver of gilthead seabream juveniles.

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NON-INVASIVE BIOCHEMICAL MARKERS FOR EVALUATING DIFFERENT FEEDING REGIMES IN GILTHEAD SEABREAM

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Introduction

Fish farming is a rapidly developing industry globally. However, the current stagnation of fish meal and fish oil production from wild fisheries hinders future growth of aquaculture. The most apparent alternatives are plant products, which have been long used in animals to minimize aquaculture's dependency on marine fisheries resources (Perera et al., 2019). Plant-derived oil and protein, on the other hand, have been shown to have deleterious impacts on the metabolism and physiology of fish due to their high non-digestible carbohydrate content and the presence of anti-nutritional elements. This gains additional importance in breeding programs to avoid compromising selection for a high growth rate in commercial environments, where several factors interact and affect growth (Xu et al., 2019). In this study, we examine the early response of blood biochemical indicators to plant-based diets in gilthead sea bream across different genetic backgrounds. The overall objective is the identification of non-invasive markers indicative of the nutritional status of gilthead sea bream.

Materials and methods

One thousand gilthead sea bream from 20 families (50 individuals per family) were tagged with an intraperitoneal chip and randomly divided into two cages, in a commercial farm of AVRAMAR SA, Greece, where they were acclimatized on a high marine-protein diet from July to September. At the beginning of the experiment in September, fish were weighed and one cage switched to a high plant-protein diet. Blood samplings were performed 15 and 30 days after diet shift. Blood was sampled by caudal puncture into heparinized syringes, transferred into tubes, and centrifuged to isolate the plasma that was stored at -80^{o}C until the quantification of cholesterol and triglycerides concentrations through colorimetric methods, and transthyretin (TTR) by novel sandwich ELISA method developed for the purpose. Thereafter, fish were weighed bimonthly until the end of production cycle in August, when the fat content was also determined.

Results and Discussion

The response to diet-shift was family-dependent. SGR differentiated with diet in certain families and only between September and November. Diet did not affect significantly fat content in any family.

Thirty days after diet shift, total plasma protein content was higher in all families feeding on the plant-rich diet and in 18/20 families it was significantly higher than in feeding on marine-rich diet. Diet significantly affected cholesterol in 9/20 families whereas 10/20 families feeding on plant-rich diet exhibited significantly higher triglyceride content 30 days after diet shift. This might be an indication of a lack of energy resources and a physiological need to access fat deposits in order to balance daily energy needs (Ibarz et al., 2007).

TTR levels were more responsive to diet and a high differentiation was observed on day 15 after diet shift. These early TTR levels were significantly correlated with the total SGR to the end of the rearing period in fish fed on the marine-rich diet as well as with the TTR levels 30 days following the diet shift. This is in agreement with earlier findings that TTR may be a potential marker of protein-energy malnutrition in gilthead sea bream (Morgado et al., 2007), and it is further reinforced with its correlation with growth performance.

In fish fed on the plant-rich diet, the total SGR to the end of the rearing period was significantly correlated with cholesterol levels 30 days following the diet shift. Also, in the same fish, a strong significant correlation between total plasma protein and cholesterol content was recorded in both sampling days. Total protein and cholesterol on day 30 were significantly correlated with SGR between September and November in marine- and plant-diet fed fish, respectively. Strong correlation among cholesterol, plasma protein and growth rate could render them representative biochemical candidates given that significant changes of them have been also reported in other diet regimes (Sitjà-Bobadilla et al., 2005).

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Conclusion

A strong interaction between genetic background and diet composition was recorded in investigating candidate non-invasive markers. Total plasma protein, TTR and cholesterol appeared indicative of the growth potential under different feeding regime. However, their response was not consistent across feeds, which calls for cautious interpretation. In any case, non-invasive biochemical markers can highlight physiological changes that occur in fish when they transition to a plant-based diet.

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MASSARRAY GENOTYPING AS A SELECTION TOOL FOR EXTENDING SHELF-LIFE OF GILTHEAD SEABREAM AND EUROPEAN SEABASS

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Introduction

Modern fish farming integrates genomics in breeding programs to guide broodstock selection and genetically select for improved offspring (Houston et al., 2020). Across-genome genetic data, such as SNPs, can be associated with a specific trait and, through parental selection, this trait can prevail in a population. European sea bass (Dicentrarchus labrax) and gilthead sea bream (Sparus aurata) are the emblematic fish species farmed in the Mediterranean (FEAP, 2021). GWAS and whole genome sequencing have led to extensive SNP discovery in the last decade (Peñaloza et al., 2021). **Improving** the shelf life of the final product is paramount to modern aquaculture towards reducing losses and total production costs. However, fresh fish is highly perishable and decay faster than other types of meat due to accelerated metabolic or structural (e.g., myofibrillar and extracellular matrix modifications) post-mortem alterations induced by endogenous proteases. In this study, we identified SNPs in genes encoding for these endogenous proteases associated with muscle deterioration and we used MassArray technology to screen commercial fish and successfully associate enzymatic activity as a trait with genetic polymorphism.

Materials and methods

A total of 161 gilthead sea bream and 186 European seabass were sampled from two commercial fish farms in Western and Central Greece, respectively. Fish were of commercial size (300-500g). White muscle samples were collected on harvest day and stored in dry ice for DNA extraction and determining enzyme activity. DNA extraction was performed using the PureLink™ Genomic DNA Mini kit according to manufacturers' instructions. DNA quality and quantity was assessed and then samples were properly diluted and sent to Inqaba Biotechnical Industries (Pty) Ltd for genotyping using the MassArray system. Whole genome sequencing was performed in pooled samples with a mean sequencing coverage of 30X on both species using the HiSeq 3000 Illumina platform. A customized bioinformatics pipeline for the analysis of FASTQ files was performed and Variant Calling Files (VCF) were generated. The activity of major proteases, namely Calpain, Collagenase and Cathepsins, responsible for white muscle degradation was measured following a protocol previously described (Ntzimani et al., 2021).

Table 1. Number of variants genotyped in European seabass and gilthead seabream. The allele frequency (%) and the difference in enzymatic activity when the allele of interest is present is also showed.

SNP ID	Gene	Alleles	Reference allele frequency %	Alternative allele frequency %	Present allele	ΔEnzymatic activity	p-value
SA_CTSDb_9	*CTSDb*	A/G	61	39	A	-14.87	6.00E-04
SA_CAPN10_11	*CAPN10*	T/A	92	8	A	-121.78	3.00E-02
SA_CAPN10_14	*CAPN10*	T/A	62	38	T	-76.26	4.40E-02
SA_CAPN2b_3	*CAPN2b*	A/T	79	21	A	-108.64	2.30E-02
SA_CAPN5a_1	*CAPN5a*	G/A	92	8	G	-109.19	3.20E-02
SA_CAPN5a_2	*CAPN5a*	A/G	86	14	G	-98.30	4.90E-02
DL_CAPN2B_1	*CAPN2B*	A/C	60	40	C	-32.23	4.70E-02
DL_CAPN14B_1	*CAPN14B*	T/A	92	8	T	-152.04	4.45E-03
DL_CAPN5b_3	*CAPN5b*	G/T	68	32	G	-47.33	2.75E-03
DL_CAPN5b_5	*CAPN5b*	A/G	67	33	G	-48.39	8.50E-03
DL_CAPN15B_1	*CAPN15B*	A/G	56	44	G	-44.57	4.20E-02
DL_Capn14b_4	*CAPN14b*	G/A	89	11	G	-56.09	1.90E-02
DL_MMP13b_1	*MMP13b*	G/A	66	34	A	-13.11	4.00E-04
DL_MMP13b_2	*MMP13b*	A/G	79	21	G	-15.05	5.80E-03
DL_MMP13a_1.1	*MMP13a*	T/C	81	19	T	-24.41	1.40E-02

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Results and Discussion

Thirty-two genes in total were identified in the genome of sea bream and sea bass encoding for calpains, cathepsins and collagenases. In total, 6,800 (*S.aurata*) and 2,608 (*D.labrax*) SNPs, were detected in those 32 genes. A filtering step was performed, selecting missense variants in genes that are expressed in the white muscle and those mapped in the catalytic/regulatory domains of the enzymes were selected. Based on these criteria, 57 and 47 missense SNPs were selected for genotyping for seabream and seabass, respectively. The association analysis revealed 6 and 9 SNPs for seabream and seabass, respectively, to be statistically significantly associated with the enzymatic activity of calpains, collagenases and cathepsins (Table 1). These SNPs combine differently in each individual, and each combination detected appears to affect enzymatic activity levels differently. These findings provide a tool for parental selection in breeding programs for extending the shelf-life of the final product.

Conclusion

MassArray can be a cost-effective tool in breeding for a specific trait. The combination of the variants arising from the current study can be used towards extending the freshness and shelf-life of these emblematic Mediterranean fish.

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THE INFLUENCE OF REARING TEMPERATURES AND LIVE FEEDS ON WHITE MUSCLE DEVELOPMENT IN GREATER AMBERJACK

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Introduction

Greater amberjack *(Seriola dumerili)* is a very promising candidate for the diversification of the Mediterranean aquaculture due to high growth rates, consumer appreciation and commercial value. Although hormonally induced spawning is well progressed, there are still many limiting factors for further expansion of greater amberjack farming [1]. High growth dispersion at hatchery stages and unsynchronized development is a major drawback in intensifying juvenile production. Temperature is a key factor in promoting fish development and growth with strong epigenetic effects [2]. Feeding at early stages is another challenging factor given the lack of a fully functioning digestive system. Rotifers and copepods are the main live feed alternatives for the early larval stages. Species of both have a great variety of amino acid and fatty acids composition, although copepods have a richer amino acid profile [3]. In this study, we investigated the influence of different rearing temperatures and different live feeds, on the process and variation of myogenesis at early development in greater amberjack.

Materials and methods

Greater amberjack eggs from a hormone-induced spawn of captive breeders were subjected to a 2X2 rearing experiment; two different rearing temperatures (20 °C vs 24 °C) were combined with two different types of live feeds [copepods (C) vs rotifers (R)] to form four experimental groups (20C, 20R, 24C, 24R). Larvae samples were collected at three developmental stages; notochord flexion (FL), end of larva rearing (ELR) and middle metamorphosis (MM). For histological analysis, larvae were fixed in cold Serra's solution and were then classified by total length (TL) by group age before chosen at random for embedding. Serial microsections (6μm) were prepared, and stained with H&E stain. Stained sections of 10 individuals per age group were examined, and images were processed using ImageJ software. The total cross-sectional area (TCSA) of white muscle in one epaxial quadrant was measured in each specimen [4]. For gene expression analysis, eight individuals per stage per condition combination (food X temperature) were sampled and stored in RNA*later* at -20 °C until RNA was isolated and cDNA synthesis was performed. The expression of 3 genes; *mylpfa* (myosin light chain 2a, hypertrophy), *mylpfb* (myosin light chain 2b, hyperplasia), *myog* (myogenin, coordination of myogenesis) were quantified by real-time PCR and normalized against two reference genes (*rps18*, *rpl13a*). Significant differences between the expression level of each gene at different conditions were analyzed using Wilcoxon signed-rank test.

Results and Discussion

Total length (TL) was almost linear after hatching and the TCSA of dorsal white muscle increased continuously between stages. The combined effect of temperature X live feed significantly affected larval growth ($p=0.037$), whereas TCSA was strongly affected by the type of live feed ($p= 0.039$). At MM, the largest dorsal epaxial quadrant was observed in the larvae reared at 24°C eating copepods (24C) ($p<0.05$). TCSA increased at different pace in the four groups with 24C exhibiting the smallest TCSA at ELR and ending with the largest at MM. Interestingly, 24C group had overall the shortest TL. Group 24R on the other hand exhibited high axial growth throughout the rearing phase. Overall, rearing at 24°C appeared to favor axial and white muscle growth.

The expression levels of *mylpfa* gene were affected neither from the temperature nor from the type of live feed. Significant differences were identified in the expression of *mylpfb* and *myog* genes between the four groups at the stages of FL and ELR. At FL, expression of *myog* that drives muscle cell differentiation and of *mylpfb* that signifies hyperplasia, was higher in larvae reared at 24°C. By ELR, group 24R exhibited the highest *myog* and *mylpfb* expression. At MM, *mylpfb* expression was highly dispersed in larvae reared at 24°C, regardless the type of live feed. The shift from larvae to juveniles is dependent on the proper deployment of hyperplastic and hypertrophic processes during the early phases of muscle development. This

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can be attributed to both temperature and diet, as the rate of hyperplastic and hypertrophic development is known to be strongly influenced by temperature during incubation and larval rearing [3]. Based on *mylpfa*/*mylpfb* expression levels, it appears that hyperplasia dominates the white muscle development up to metamorphosis and it is strongly affected by rearing temperature.

Conclusions

Both rearing temperature and feed type influenced white muscle development in greater amberjack larvae. The combined results from histological and gene expression analyses indicate that temperature is the major factor with 24ºC favoring a higher pace of white muscle development in comparison with 20ºC. Within 24ºC, the type of live feed used resulted in different phenotypes at MM with rotifers supporting longer larvae with smaller TCSA as compared with copepods. A higher development pace was accompanied by a higher variation in gene expression levels that were not accompanied by higher variation in phenotypic traits, i.e. TL and TCSA.

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OCCURRENCE OF MYCOTOXIN IN AQUA FEED SAMPLES AND RAW MATERIAL IN 2022

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Mycotoxins are secondary fungal metabolites which can affect health and performance of aquatic specues and are frequently found in various feedstuffs. Mycotoxin occurrence and contamination levels in fish and shrimp feed were monitored in this study. Due to the natural co-occurrence of mycotoxins, the toxicity of contaminated feed cannot be accurately estimated by determining the concentration of one only. The following study represents an effort to further broaden the knowledge on mycotoxin occurrence and co-occurrence in feed.

A total of 110 fish and shrimp feed and feed raw materials samples were analyzed using Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS) for six major mycotoxin groups: aflatoxins, type A trichothecenes (DON, NIV, ..), type B trichothecenes (T-2, HT-2, ..), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A.

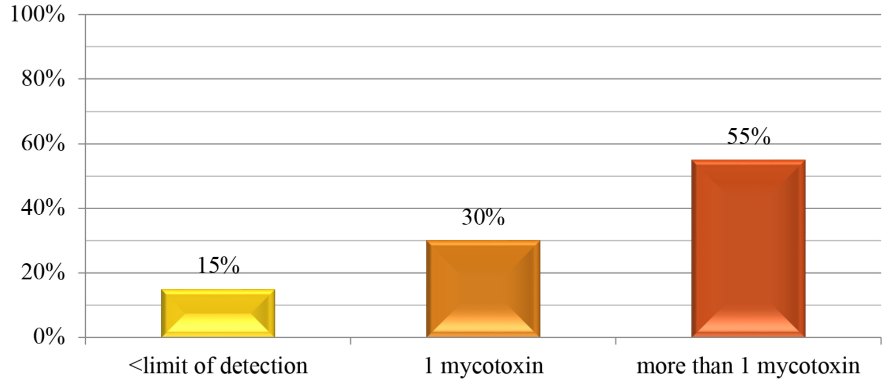
One of the most common fungal metabolites detected was deoxynivalenol (detected in 55% of samples, with an average concentration of positive samples of 239ppb and a maximum concentration of 710ppb). Other common mycotoxins included fumonisins (50% of samples, mean 379 ppb, max 3837 ppb) and zearalenoe (38% of samples, mean 31 ppb, max 208 ppb). 23% of samples showed DON and ZEN co-occurrence whereas 28% showed co-occurrence of DON and FUM, all 3 mycotoxins are produced on the field by *Fusarium* species.

Thanks to the increased sensitivity of mycotoxin analysis a greater number of mycotoxins and fungal metabolites have been reported. The potential for multi-mycotoxin contamination validates use of multiple mitigation strategies to properly address animal health and performance concerns.

All Regions - Table of Mycotoxins Detected including Risk Threshold

Total Risk Level: 45%	**Afla**	**ZEN**	**DON**	**T-2**	**FUM**	**OTA**
Number of samples tested	110	108	110	76	110	76
% with mycotoxin detected	24%	38%	55%	4%	50%	28%
% above health risk threshold	12%	6%	29%	0%	11%	3%
Average of positive (ppb)	98	31	239	25	379	5
Median of positive (ppb)	2	22	211	21	235	3
Maximum (ppb)	2194	208	710	48	3837	38
Recommended max threshold (ppb)	2	50	150	50	500	10

Co-contamination of mycotoxins on All Samples - samples tested for at least 3 mycotoxins



OBSERVATIONS ON TANK SPAWNING OF PIKEPERCH FEMALES WITHOUT MALE COMBINED WITH OVARIAN LAVAGE

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Introduction

The development of pikeperch (*Sander lucioperca*) production has accelerated over the last 20 years, thanks to significant R&D efforts. In addition to traditional pond production, the growth of intensive farming is also striking. The first step is the development of the controlled and programmed reproduction. At present, hatchery breeding using *in vitro* fertilisation (collection and dry fertilisation of the gametes) is the most researched area. In our previous studies, we demonstrated that sperm injected into the ovaries of externally fertilized fish retains its fertilizing ability several hours before induced ovulation. This method can be successfully used in combination with (Müller et al. 2018, 2020) or without hormonal treatment (Gazsi et al. 2021) in case of several species. The aim of our study was to investigate the efficiency of sperm ovarian lavage in case of pikeperch induced spawning in the absence of male. Additionally, in order to assess the appropriate moment for the lavage, we monitored the effect of carp pituary (CP) suspension on final oocyte maturation and ovulation.

Materials and methods

The pikeperch broodstock was transported from the Nagyatád V95 LtD fish farm (Nagyatád, Hungary) to the Laboratory of MATE Kaposvár Campus. The fish were sorted by sex (10 females, 4 males) and individual females were separated in 250 l tanks, while male specimens were placed in pairs into two 300 l tanks. The tanks were in same water circuit and the water temperature was maintained between 13.2-14.7°C. Hormone treatment of singular injection of 7 mg/kg CP was administrated intraperitoneally. The maturation stages of oocytes (Zarski et al., 2012) were assessed following biopsy sampling of all females at three different moments - at the same time as hormone treatment (0 h), at 48 h and 70 h post-injection. Clarified oocytes were photographed and oocyte stage was evaluated. Artificial nest substrate (60×70 cm) was inserted for spawning, straight after sperm ovarian lavage. The method of sperm injection was as described by Müller et al. (2020). Briefly, 1 ml sperm per kg of female's body weight was equally divided into two ovarian lobes 75 h after hormone treatment. The timing of sperm injection was determined based on the oocyte stage and previous studies so that the "ovarian storage time" would not exceed 40 h, the time when spermatozoa fertility greatly reduces (Müller et al., 2020).

Results

In total nine fish ovulated and released their eggs. The maturation dynamics of the 9 females showed large individual differences, visible in latency time being at 83h (n=1), 88h (n=1), 95h (n=5), 99h (n=1), 154 h (n=1). Several fish did not ovulate on the artificial substrate but disposed the eggs next to it (n=4). Fertility values measured at 72 h post-fertilisation showed large individual differences in total range 0-84%. There was no correlation between the fertilisation rate and ovulation time (sperm storage time).

Discussion

The ovarian lavage method was successfully used with different siluriform and cypriniform species (Müller et al., 2018, 2020; Gazsi et al, 2021). To the best of our knowledge, this is the first report on the application of this fertilisation method in pikeperch. The outcome of the technique appears to be rather variable, nevertheless, such variability was already described in pikeperch on different issues (Ljubobratović et al., 2017, Zarski et al., 2019; Colchen et al., 2020). Therefore, future studies are required to adapt this method to pikeperch and other perciforms respecting the biology of their gonads.

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Acknowledgements
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MAXIMISING BIOMASS GAIN AND FEED CONVERSION EFFICIENCY IN RECIRCULATING AQUACULTURE SYSTEMS USING A NEW REAL-TIME NITRITE AND NITRATE MONITOR

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Introduction

Recirculating aquaculture systems (RAS) have the potential to reduce fish stress and improve welfare by controlling a range of parameters including feed rates and water quality [1]. RAS has been employed in freshwater phase of Atlantic salmon production with ongoing investigations to produce larger pre-smolts to reduce the required length of time of the marine grow-out phase [2]. To ensure successful production, nutrient balance and biofilter performance in RAS systems is key. Monitoring of nitrite (NO_2^-) and nitrate (NO_3^-) aims to maximise biomass gain and feed conversion efficiency in RAS. Current methods for nitrite analysis include spectrophotometry, however this methodology consists of time-consuming sample preparation and uses hazardous reagents. Instead, the use of Aquamonitrix real-time nitrite and nitrate analyser can overcome these drawbacks while allowing to monitor the correct operation of the biofilter; manage water quality and optimise feed conversion efficiency.

The aim of this case study was to identify optimal feed rates and water quality and achieve maximum feed conversion efficiencies. Aquamonitrix nitrite and nitrate monitor was used. At the start of the case study, monitoring of nitrite and nitrate levels ensured bacterial communities in biofilter had established and ammonia was oxidised to nitrite and then nitrate. Real-time results provided by Aquamonitrix informed of water quality in the RAS system.

Methods

The case study was carried out in the RAS facility of the Atlantic West coast of Ireland (operated by the Marine Institute). The system has eight 5.5 m^3 tanks with a total 44,000 L capacity and production capacity of 12,000 Atlantic salmon pre-smolts in the freshwater phase. Feeding was daily, starting at 8.30 am, with 20.4 grams added to the system every 90 seconds for 12 hours.

In-situ monitoring was carried out using Aquamonitrix nitrite and nitrate analyser. The analyser incorporates rapid ion separation in combination with novel UV LED based detection for selective and sensitive detection of nitrite and nitrate in waters [3]. Real-time nitrite and nitrate concentrations were remotely accessible and reported via the proprietary Datamonitrix IoT portal which allows users to remotely access continuous concentration information generated by the analyser deployed within the RAS facility.

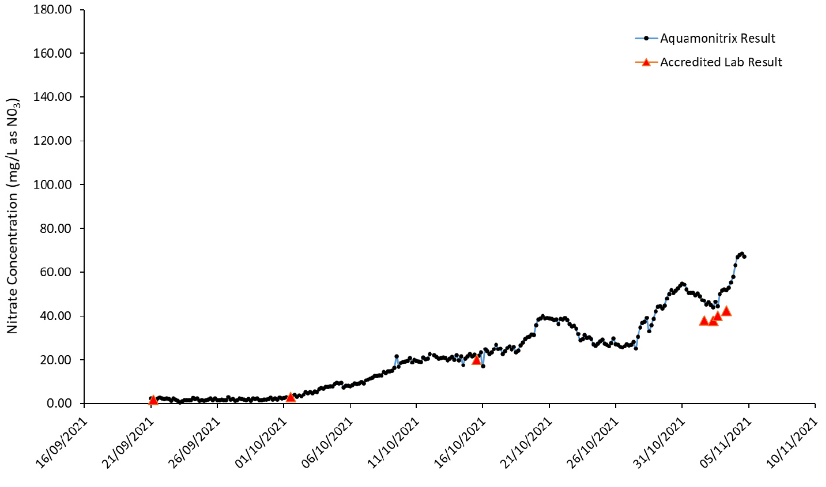

Figure 1: Nitrate accumulation in the system overtime. Aquamonitrix results were compared to accredited laboratory results to demonstrate accuracy of the unit on the monitoring of nutrients.

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Results

Temporal resolution of results provided by Aquamonitrix allowed the observation of fluctuations of nitrite levels in relation to feed input through the day. Nitrite build-up started after the feeding started and continued during the day. This nitrite build-up was then reduced overnight as bacteria broke it down to nitrate.

Nitrate accumulation was also detected in the system overtime (Figure 1). Nitrate increased along with increasing fish biomass. Biomass gain and total feed input were closely correlated with a linear increase of these factors.

Discussion

Results obtained from this case study using Aquamonitrix for the monitoring of nitrite and nitrate in a RAS system showed the importance of specific and accurate nutrient monitoring with high temporal resolution. The observed nitrite fluctuations aligning with feeding cycles within a single day highlight the speed at which real-time results can be obtained. The monitoring of these nutrients also provides the correct operation of the biofilters, in this case observed by the decrease of nitrite overnight which is being oxidised by bacteria present in the biofilter. Correlations between nutrients and fish biomass allow to determine water exchange rates and track water quality in the system. It was concluded that efficient feed conversion occurred in the RAS system, emphasising the environment in which the fish were present was of high-water quality. The operation strategy of the RAS system to achieve the optimal environment of efficient feed conversion was obtained through the high temporal and accurate monitoring nitrite and nitrate using Aquamonitrix analyser.

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AQUACULTURE STEWARDSHIP COUNCIL (ASC) CERTIFICATION: EVALUATION OF NICHE SPECIES (I.E. PERCH AND CAT FISH) FOR THE DEVELOPMENT AND IDENTIFICATION OF SPECIES AND SYSTEM-SPECIFIC CERTIFICATION METRICS

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The Aquaculture Stewardship Council is the world's leading certification scheme for farmed seafood. The ASC uses market-based approaches to incentivise famers to achieve strict standards regarding environmental and social performance. Currently, the ASC standards cover 17 species groups under 11 standards, plus a joint ASC-MSC standard for seaweed. Standards development to date have been multi-year dialogue processes involving many stakeholders from academia, producers, ENGOs, and others. While these processes have resulted in robust standards, they are lengthy and do not allow for a quick response to new or emerging species in the market. To respond to requests more quickly for new species standards, the ASC has developed a niche species process.

Developing alongside our Farm Standard process, the niche species project recognizes that many impacts of aquaculture are based on the production system, rather than only being species dependent. The Farm Standard will include a core set of indicators and will monitor ecosystem impact consistently across species and regions and set species-specific limits where necessary (e.g., mortality levels, feed conversion, etc.). Therefore, this is the perfect time to create a process to add species and framework more easily for developing metrics.

The niche species development itself begins with an analysis of market demand and emerging species in aquaculture production. Priority species are then entered into a risk matrix, which identifies areas of environmental or social concern. For example, species that are highly invasive, require wild caught broodstock, or which present concerns from a human welfare perspective will be flagged. While not necessarily meant to eliminate these species from consideration, the risk matrix will highlight areas which may require further investigation or consultation. A list of potential species for inclusion will then be reviewed by the ASC Supervisory Board, who provide the final decision on which species shall be developed.

The ASC is currently partnering with catfish and pikeperch producers in Europe to gather data and develop metric limits for these species. Pilots will be conducted with these farms to test the process, after which a formal methodology can be released.

The combination of the Farm Standard and niche species project will improve the ASC in several ways. First, the volume of aquaculture production able to be certified will increase with each additional species added. Second, a consistent set of standards across species will allow for us to better determine impact. The development of improved reporting templates outlining metrics requirements across species will allow for trend analysis and robust impact reporting.

EFFECT OF PROCESSED (BIOSECURE) MACROALGAE ON GROWTH AND HEALTH OF ABALONE *Haliotis midae*

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Introduction

The provision of microbiological safe diets is a crucial aspect in any intensive culture condition. This reduces the risk of introduction of potential pathogens to the stock which could lead to infections and disease outbreaks. In the abalone industry, macroalgae is often harvested from the sea or grown in abalone farms in the integrated multi-trophic aquaculture (IMTA) systems then fed directly to the farmed abalone stocks or incorporated into formulated abalone diets. Despite the benefits of macroalgae inclusion on abalone growth, microbial hazards linked to the use of fresh macroalgae have been reported (Kua et al. 2011, Banach et al. 2020, Barberi et al. 2020). Application of biosecurity processing treatments to render the macroalgae safe is recommended as a necessary step for their effective utilization. However, there is limited information regarding the effect of processed macroalgae on the fed abalone stocks. In this study, three macroalgae *Ecklonia maxima*, *Ulva lactuca* and *Gracilaria gracilis* were subjected to a combination of different biosecurity processing treatments: disinfection with povidone-iodine solution, oven drying and UV-C treatment before being incorporated into formulated abalone diet. The effect of the biosecure macroalgae meal on the growth performance and health of abalone was assessed.

Materials and methods

Seven experimental diets were formulated and fed to sub-adult abalone for a period of five months. Three diets contained biosecure macroalgae kelp, gracilaria and ulva respectively, other three contained the same macroalgae but were not subjected to any biosecurity treatment, the remaining diet was the control and did not contain macroalgae. Diet formulations were conducted according to standard commercial diet formulation. The seven diet treatments were fed to baskets of abalone in four replicates using a randomized-block design with each treatment represented once in each tank to ensure standardized conditions. Feeding was carried out using standard farm feeding procedures of 0.3-0.4% of body weight per day. Sampling was done at the start of the experiment, after one month, at month four and end of the experiment. Total weight of the abalone baskets was taken using an electronic balance after draining off the excess water. Sub-samples of thirty abalone were then taken randomly from each basket, blotted dry to remove excess water then individual shell length and weight taken. Weight was recorded to the nearest 0.01 g using a balance while shell length was measured along the longest axis to the nearest 0.01 mm using Vernier callipers. To assess the health indicators, two whole abalones were taken from each replicate basket at the end of the experiment for histopathology analysis.

Table 1: Mean (± se) wet weights and growth rates of abalone *Haliotis midae fed with different diets containing biosecure and non-biosecure macroalgae: Abfeed* (AB) biosecure gracilaria (BG), non-biosecure gracilaria (NBG), biosecure ulva (BU), non-biosecure ulva (NBU), biosecure kelp (BK) and non-biosecure kelp (NBK). (preliminary data)

Diet Treatments	Mean Initial Weight (g ± se)	Mean Final Weight (g ± se)	Growth Rate (g day^{-1})
AB (control)	37.54 ± 0.48	53.04 ± 1.02	0.101
BG	37.98 ± 0.48	56.55 ± 0.8	0.121
NBG	37.64 ± 0.51	57.41 ± 0.81	0.134
BU	37.92 ± 0.53	56.99 ± 0.81	0.127
NBU	37.65 ± 0.46	59 ± 0.87	0.139
BK	37.8 ± 0.43	57.58 ± 0.96	0.129
NBK	37.46 ± 0.48	55.27 ± 0.74	0.116

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Results

Preliminary results indicate that, overall, the macroalgae containing diets produced significantly higher growth compared to the control. Diet containing non-biosecure ulva (NBU)recorded the highest growth followed by non-biosecure gracilaria diet (NBG) (table 1). There was a significant difference between treatments in terms of final abalone weights ($p<0.05$). Analysis to determine the effect of the biosecure diets on health of abalone is ongoing.

The findings of this study are expected to contribute towards improved growth and health of farmed abalone stocks, and a reduction in the risk associated with diseases and pathogens that could potentially be introduced to abalone stock through feeding practices.

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ARE FISH LARVAE FULLY IMMUNOCOMPETENT AT EARLY STAGES?

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Introduction

Early development is a critical stage since the highest mortality of fish occurs in this period (Garrido et al., 2015)the relation between the size-at-hatch and early survival was assessed using laboratory experiments and on field-caught larvae of the European sardine (Sardina pilchardus. The high vulnerability of fish to disease and stressors during larval stages compared to post-larval stages raises a question about the immune capacity of fish larvae before and after the larval - juvenile transition. Overall, the immune repertoire of teleosts and presumably the immune response is more complex than in other vertebrates because of the acquisition and retention of additional gene copies during evolution of immune-related genes as exemplified by the complement genes (Najafpour et al., 2020)factor H (Cfh. Studies of immune-related molecules during larval development tend to take a candidate gene approach, but little information is available about the acquisition of full immune capacities in fish. For example, in the Atlantic cod until 8–10 weeks post-hatching IgM was absent (Schrøder et al., 1998), which suggests the immune repertoire and immune response may be different in early developmental stages. Wang et al. (2008) studied the differential expression of several complement genes, C3, C1r/s, C4, C6, Bf, MBL and MASP in zebrafish (*Danio rerio*) and proposed the alternative pathway is active and responded to LPS in larval stages. Gilthead sea bream (*Sparus aurata*) is an important commercial marine species, and disease management and production of high-quality larvae of this species remains a challenge for the aquaculture industry (Muniesa et al., 2020)risk analysis and biosecurity systems. Mediterranean marine fish farming, as any aquaculture production, is affected by various infectious diseases. However, seabass and seabream, the main produced species, are not listed as susceptible host species for the notifiable pathogens listed in the current EU legislation, which generates a lack of systematic reporting. The results presented in this study come from a survey directly to fish farms (50 hatchery and on-growing units from 10 Mediterranean countries. In the present study, the goal was to use high-throughput sequencing (RNA-seq) of sea bream larvae during flexion and at mid-metamorphosis to prospect the potential immune capacity of fish larvae during very early life stages.

Material and methods

Total RNAs (tRNA) of the whole larvae in flexion and mid-metamorphosis stages were extracted using an E.Z.N.A. Total RNA Kit I (VWR, USA) according to the manufacturer's instructions. Initially pools composed of 3 sea bream larvae at flexion and 1-2 larvae at mid-metamorphosis were homogenized in lysis buffer using mechanical disruption with two iron beads (5 mm) and a Tissue lyser II (Qiagen, Germany) for 3 cycles (30 Hz) of 30 seconds at room temperature. Paired-end RNA-seq sequencing libraries (2 × 150bp read length) were produced using an Illumina HiSeq xten platform (Novogene, Shanghai, China). After quality control filtering, the reads were mapped to the seabream reference genome (https://www.ncbi.nlm.nih.gov, RefSeq assembly accession: GCF_900880675.1) using the bioconductor package Rsubread (Liao et al., 2013). Analysis of differentially expressed (DE) genes was performed using normalized read counts and by computing moderated t-statistics of differential expression by empirical Bayes moderation of the standard errors in the limma package (Ritchie et al., 2015)limma has been a popular choice for gene discovery through differential expression analyses of microarray and high-throughput PCR data. The package contains particularly strong facilities for reading, normalizing and exploring such data. Recently, the capabilities of limma have been significantly expanded in two important directions. First, the package can now perform both differential expression and differential splicing analyses of RNA sequencing (RNA-seq.

Results

The expression profile of the immune genes involved in both innate and adaptive immunity was analyzed in sea bream at flexion and mid-metamorphosis. Transcriptome comparisons of the two stages of sea bream larvae identified 150 immune-associated gene transcripts that were significantly changed between flexion and mid-metamorphosis. Overall, most of the immune-associated genes involved in innate and adaptive immunity were down-regulated in younger larvae at the flexion stage (≅ 24 dph) compared to the bigger larvae at mid-metamorphosis (≅ 50 dph). Several genes associated with the adaptive immune response such as C1q (e.g., *c1qa*, *c1qb*, *c1qc*) and MHC (e.g., several *mr1*) were down-regulated in the flexion stage. Gene ontology of the DE genes in 24 dph sea bream larvae (compared to 51 dph) showed enrichment of "*Abnormality of complement system*" (HP:0005339) and "*Complement deficiency*" (HP:0004431) terms based on the human phenotype ontology (HPO). Many immune-associated genes were expressed in both flexion and mid-metamorphosis larval

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stages while some immune gene transcripts such as MHC (e.g., major histocompatibility complex class I-related gene protein-like, XM_030411748), C1q (e.g., complement C1q-like protein 4, XM_030431421) and lectin (e.g., galactose-specific lectin nattectin-like, XM_030422915) were absent at the flexion stage (≅ 24 dph) compared to mid-metamorphosis (≅ 50 dph).

Discussion and conclusion

The significant change in many immune-related gene transcripts between larvae at flexion and mid-metamorphosis suggests that the immune capacity of sea bream larvae changes during development. The clear down-regulation of most of the immune-associated gene transcripts in larvae at flexion is indicative of a very low immune capacity in very early stages of sea bream. This is coherent with the observed increase in a suite of immune-associated genes (C3, C1r/s, C4, Bf, MBL and MASP) in zebrafish larvae after hatching (Wang et al., 2008). The lack of immune competence in early life stages leading to severe mortality was also proposed for the Indian major carp, *Labeo rohita*, which had low mean antibody levels up to 3-weeks post-hatch (Swain et al., 2006)Labeo rohita (Ham.. Most innate immune genes showed an increase in transcription around hatching and first feeding in Atlantic cod, *Gadus morhua* (Seppola et al., 2009). Taking into consideration the results about complement in our transcriptome study of sea bream larvae and the study of the zebrafish complement response where the competency of the alternative pathway was suggested (Wang et al., 2008), it seems likely that activation of complement by the classical and lectin pathways does not occur in early development. The absence of gene transcripts related to the adaptive response at flexion in sea bream and their upregulation at mid-metamorphosis (≅ 50 dph) suggests the adaptive response emerges later in the development of sea bream larvae. Overall, the transcriptome data suggests that although many gene transcripts related to the immune system are present in the flexion stage, levels are very low and the larvae are unlikely to be immunocompetent. The levels of immune-related gene expression increased during development and achieved significantly higher expression at mid-metamorphosis. The results of this study about the immune status of sea bream larvae as revealed by their transcriptome will contribute to improve the management and efficacy of immunostimulants and vaccines.

Acknowledgements

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EVALUATION OF DIETARY NUCLEOTIDES AND NUCLEIC ACIDS ON GROWTH AND GUT HEALTH OF EUROPEAN SEA BASS (*European seabass*) JUVENILES FED DIFFERENT FISHMEAL LEVEL

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Introduction

Nucleotides (NT) and nucleic acids (NA) are considered promising additives in aquafeed formulation. Dietary NT and NA have been shown to enhance growth, improve disease resistance, modulate immune functions and positively affect the gut microbiota of many fish species (Hossain et al., 2020). NT and NA could be beneficial in-feed additives for fast grower carnivores fish species such as European seabass (Dicentrarchus labrax). Moreover, NT and NA may enhance the expression of certain genes which are important for non-specific defences (e.g. cytokines) as well as increasing the resistance to a variety of stressors. The objective of this study was to evaluate the effects of dietary nucleotides and nucleic acids on growth of European sea bass juveniles fed different fish meal level.

Material and Method

Six isoproteic and isoenergetic experimental diets formulated to contain two different fish meal (FM) levels (FM10, 10 % and FM20, 20%) were added with 500 ppm of NT or 500 ppm of NA (FM10, FM10NT, FM10NA, FM20, FM20NT, FM20NA). Diets were tested on triplicate fish groups of 60 individuals (initial weight: 14.33 g) over a period of 88 days. During the trial, feed was provided to apparent satiation, temperature was kept at 23.0 ± 1.0 °C and oxygen above 7.5 mg L^{-1}. At the end of the trial, fish were exposed to suboptimal conditions by increasing temperature to 30.0 ± 0.5°C and decreasing oxygen to 4.5 mg L^{-1} for 8 days while keeping the same feeding conditions. Final body weight, specific growth rate (SGR), feed intake (FI) and feed conversion rate (FCR) were calculated. Intestine and liver cytokine gene expression, as well as gut microbiome were performed before and after the exposure to suboptimal rearing conditions. Differences among treatments were considered significant at $P < 0.05$.

Results

At the end of the trial fish fed diets with NT and NA inclusion presented significantly higher body weight. Fish fed FM20NA presented the highest body weight and SGR and the lowest FCR. FI has given significant difference. Feed with NT and NA inclusion gave higher palatability. Concerning somatometric indices, no differences occurred among groups. On proximate composition, fish fed FM10, FM10NA and FM20 displayed most elevated water body retention. Higher crude lipid retention occurred in FM20NT group. On nutritional indices, FM20NA diet showed the most elevated protein and lipid efficiency (PER, LER, GLE, GPE). All cytokines tested (IL-1 β; TNF-α; IL-10; TGF-β) showed a significant effect on treatments. Comparison between groups at the end of the feeding trial (T1; Fig. 1) showed that the gene of the pro-inflammatory IL-1β cytokine was significantly more expressed ($p = 0.0260$) in fish with FM10NA compared to fish with FM20NT. IL-1β was significantly higher in fish with FM10NA ($p = 0.0260$) or FM10NT ($p = 0.0043$) compared to fish fed with FM20NA and FM20NT. Conversely, the gene expression of the regulatory TGF-β cytokine was significantly higher in groups with FM20NA ($p = 0.0043$) or FM20NT ($p = 0.0043$) compared to group fed with FM10NA and FM10NT. No significant effects were detected in fish fed with FM10/FM20. After the suboptimal rearing condition period (T2; Fig. 1) there was a significant difference ($p = 0.0411$) in the gene expression of the pro-inflammatory cytokine TNF-α. The FM20NA group showed the highest TGF-β expression with values significantly higher than FM10NA ($p = 0.0303$) and FM20NT ($p = 0.0411$).

Discussion and conclusion

This study provided novel insight on the effects of NT/NA inclusions for European seabass juveniles fed different FM-FO levels. It improved voluntary feed intake by 10.7% at FM10 and 9.4% at FM20. An increase in SGR was observed with each inclusion of NT/NA in the diet compared to the only FM diet. Notably, fish fed with NT/NA inclusions in the FM20 diet showed a marked improvement in lipid and protein absorption. NT/NA inclusions in the FM20 diet have had a positive impact on the intestinal mucosa by increasing the anti-inflammatory TGF-**β**. Thanks to the increase in TGF-**β** they had a better nutritional absorption resulting in improved growth performance. In conclusion, results on growth performance, nutritional indices and cytokines gene expression suggest the addition of NT and NA in the diet of European sea bass (*Dicentrarchus labrax*) juveniles.

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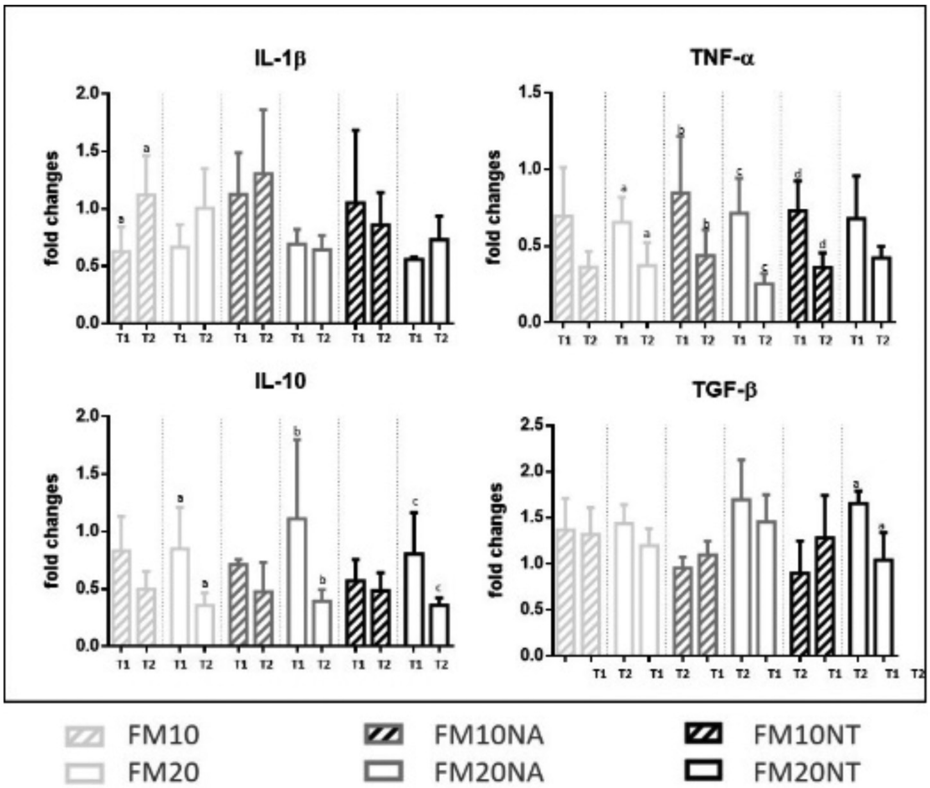

Figure 1. Gene expression analysis at the end of the feeding trial (T1) and after the suboptimal rearing condition period (T2). Comparison between different time points (T1 *vs* T2): same superscript letter means a significant difference ($p \leq 0.05$).

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UNRAVELING THE POTENTIAL OF MARINE INVERTEBRATES AS A SOURCE OF OMEGA-3 LONG-CHAIN POLYUNSATURATED FATTY ACIDS FOR AQUACULTURE

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Introduction

In the current context of rapid growth, marine aquaculture faces a major challenge for sustainable expansion given its economic and environmental dependence on the limited availability of marine-derived meals and oils used in feed formulation. This linear model is unsustainable and therefore, the current aquaculture of marine species is based on the use of replacement feed with high levels of inclusion of plant components whose nutritional profiles are far from those of marine ingredients. Specifically, the use of vegetable oils entails a drastic reduction in n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), absent in vegetable oils, and a concomitant increase in other shorter (up to 18 carbons) polyunsaturated fatty acids (PUFA) (Naylor et al., 2009), including linoleic (18:2n-6) and linolenic acids (18:3n-3). Certain aquatic invertebrates have the capacity for the endogenous production (biosynthesis) of n-3 LC-PUFA. This feature became apparent following a study (Kabeya et al., 2018) that revealed that a particular type of enzymes ("ωx desaturases"), historically believed to be absent in animals, exist in multiple invertebrates. The presence of omega desaturases gives these invertebrates the potential to biosynthesize n-3 LC-PUFA, just like the primary producers. Such metabolic capacity, together with their detritivorous feeding habit, make some aquatic invertebrates ideal candidates to apply the principles of the Circular Economy, through the recycling of waste materials of low nutritional value from bioindustries, to produce a highly nutritious biomass. This can represent a new sustainable source of n-3 products in high demand by rapidly expanding sectors such as aquaculture, and can contribute to transform the aforementioned linear model into a circular one. In this context, different national and international projects (IMPROMEGA, SIDESTREAM, BIOCYCLES) are being developed to delve into these aspects, using polychaete annelids, and gammarid and copepod crustaceans. The ultimate objectives pursued include: 1) to investigate the in *vitro* and *in vivo* capacity for the biosynthesis of n-3 LC-PUFAs; 2) to evaluate the regulation of the n-3 LC-PUFAs biosynthesis through dietary lipids; 3) to study the regulation of the enzymes involved in the biosynthesis of n-3 LC-PUFAs through environmental factors.

Materials and Methods

The generic approach to address the objectives consists of the selection of species, followed by the molecular and functional characterization of the enzymes involved in the biosynthesis of fatty acids, basically elongases and desaturases. Molecular characterization is based on the search for sequences in both public (e.g. NCBI, Ensembl) and non-public genomic and transcriptomic databases accessible through collaborations. The sequences are then used for the design of degenerated primers to obtain the complete sequences of the genes of interest by means of PCR. The functional characterization is based on the heterologous expression of the genes in a yeast system (*Saccharomyces cerevisiae*). The species that so far have been studied are the polychaetes *Hediste diversicolor* and *Platynereis dumerilii*, the gamarid crustaceans *Echinogammarus marinus* and *Gammarus locusta*, and the copepods *Tigriopus californicus*, *Apocyclops panamensis* and *Platychelipus littoralis*.

Results and Discussion

Polychaetes have **ω**x desaturases and a complete enzymatic set that theoretically allows them to carry out the *de novo* synthesis of LC-PUFAs, mainly eicosapentaenoic (20:5n-3) and arachidonic (20:4n-6) acids, but also docosahexaenoic acid (22:6n-3), although the latter to a lesser extent (Kabeya et al., 2020). As for the gammarids elongases, but not desaturases have been found. This limits their theoretical capacity for LC-PUFAs synthesis, although recent studies show their potential to be cultured from agriculture and other bioindustries residues while maintaining moderate levels of LC-PUFAs (Ribes-

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Navarro et al., 2021, 2022). In copepods, the enzymatic capacity seems to be phylogenetically driven, being complete or near-complete in the Harpacticoids, intermediate in the Cyclopoids and limited in the Calanoids. Species such as *T. californicus* can be net generators of LC-PUFAs like the primary producers (phytoplankton and other microorganisms) (Kabeya et al., 2021). The real enzymatic capacity of invertebrates for the biosynthesis of n-3 LC-PUFAs should be investigated through feeding experiments designed to provide complementary data to those already obtained *in vitro* and that will allow to individually characterise the expression and function of enzymes involved in the n3 LC-PUFAs biosynthesis. This is underway in our laboratories, and is a fundamental step to understand how the use of metabolic precursors contained in waste materials (sidestreams) used as food, can be optimized by manipulating culture conditions, and thus study how diets with poor lipid profiles similar to those of the sidestreams of bioindustries such as aquaculture and agriculture, can modulate the biosynthetic capacity of n-3 LC-PUFAs (nutritional regulation). Apart from the diet and its lipid composition, environmental factors such as temperature and salinity are implicated in the regulation of the genes involved in the biosynthesis of n-3 LC-PUFAs, which offers the opportunity to develop culture protocols aimed at a greater production of these compounds and avoiding or, where appropriate, minimizing genetic manipulation.

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ANTIMICROBIAL RESISTANCE IN INDICATOR BACTERIA FROM A RECIRCULATING AQUACULTURE SYSTEM AND COMPARISON WITH CLINICALLY IMPORTANT ISOLATES

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Introduction

Aquaculture is considered a hotspot for the emergence and spread of antimicrobial resistance (AMR) because the coexistence of fish, bacteria and antibiotics in the aquatic environment provides the ideal conditions (Cabello et al, 2013). However, little is known on AMR in aquaculture compared to terrestrial farming. In particular, studies on the occurrence of AMR in recirculating aquaculture systems (RAS) are very scarce, in spite of being the fish farming system of the future. The objectives of this study are 1) to describe the dynamics of selected indicator bacteria within a RAS (i.e. sources, persistence and diffusion) and 2) to compare their AMR profiles to those from clinically important isolates in human medicine.

Materials and methods

This project is being performed at the RAS based at the vet school Oniris, Nantes, over six months (February-July 2022). Rainbow trout is farmed year-long at this experimental station that uses domestic water and follows a thorough water treatment: sand filtration, biological filtration and UV. The dynamics of AMR within this RAS are being studied with a longitudinal study design, by tracking a selection of indicator bacteria in all compartments (water, sediment, fish, feed and biofilm). On a monthly basis, the following samples are collected: 7 water samples from different parts of the recirculating system (including each water treatment step, see Table 1), 1 sediment from water reservoir, 5 biofilms and 5 fecal samples from fish tanks. Each batch of fish feed distributed during the study period is sampled once. Dead fish are opportunistically sampled. All the samples are tested for the presence of *Escherichia coli*, *Pseudomonas* and *Aeromonas*. *E. coli* is an indicator of fecal contamination from mammals and birds, whereas *Aeromonas* and *Pseudomonas* are ubiquitous aquatic bacteria. All three bacterial groups are good indicators of AMR in the environment as they are prone to accumulate and exchange AMR genes. Samples are plated on Glutamate Starch Phenol Red agar (Merck, Germany) for detecting *Aeromonas* and *Pseudomonas* and on Eosin Methylene Blue agar for detecting *E. coli* (Biokar, France), then incubated at 37° C for 24-48 hours.

MALDI-TOF will be used to confirm and identify isolates at species level. Confirmed isolates will be tested by the broth micro-dilution method to determine their Minimum Inibitory Concentration. The antibiotics used in this antimicrobial susceptibility test include Sulfamethoxazole, Trimethoprim, Ciprofloxacin, Tetracycline, Meropenem, Azithromycin, Nalidixic Acid, Cefotaxime, Chloramphenicol, Tigecycline, Ceftazidime, Colistin, Ampicillin, Gentamycin and Amikacine. For each of the bacterial groups studied (*E. coli, Aeromonas, Pseudomonas*), 20 isolates from human clinical infections have been retrieved from the collection of the University Hospital (CHU Nantes) for comparison of AMR profiles.

Results and discussion

44 samples have been tested so far in two sampling events. *Aeromonas* is presumptively the most prevalent bacteria, being found in all compartments of the RAS and almost all the samples. *Pseudomonas* on the other hand seems less prevalent, especially in fish feces. No *E. coli* was found, but coliforms were isolated from water, sediment, biofilm and feed. Further analyses are needed to know whether coliform contamination from feed does persist in this RAS.

With regards to water, only domestic water is presumptively free of *Aeromonas* and *Pseudomonas*, meaning that water is the cleanest at the arrival to the station (Table 1). Fish and feed would be the main sources of these bacteria, which are likely to persist in this RAS. Interestingly, biofilm seems the most dynamic compartment of this system, presumptively shifting from being mainly *Aeromonas*-positive to being mainly *Pseudomonas*-positive, as water temperature rose from 14°C to 16°C.

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Table 1: Preliminary results. Type of water and number of samples suspected to be *Aeromonas*- and *Pseudomonas*-positive

Type of water (n° of samples)	**Number of samples presumptively positive for *Aeromonas***	**Number of samples presumptively positive for *Pseudomonas***
Domestic (2)	0	0
After sand filtration (2)	2	1
After biological filtration (2)	2	2
After UV (2)	2	2
Before arrival to fish tanks (2)	2	2
Water reservoir (2)	2	2
All water samples (12)	**10**	**9**

Table 2: Preliminary results. Proportion of suspected *Aeromonas*- and *Pseudomonas*-positive samples by sample type and month. Water temperature at the time of sampling is shown.

	February (14° C)		**March (16°C)**	
Sample type	**Number of samples presumptively positive for *Aeromonas***	**Number of samples presumptively positive for *Pseudomonas***	**Number of samples presumptively positive for *Aeromonas***	**Number of samples presumptively positive for *Pseudomonas***
Water	4/5	3/5	6/7	6/7
Sediment	1/1	1/1	1/1	1/1
Fish feces	9/10	4/10	7/7	2/7
Biofilm	5/5	0/5	2/5	4/5
Feed	3/3	3/3	Same feed batch distributed	

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ANTIMICROBIAL-RESISTANT *Escherichia coli* IN THE RAINBOW TROUT FARM ENVIRONMENT IN BRITTANY, NW FRANCE

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Introduction

Antimicrobial resistance (AMR) is a threat for human and animal health, and for the production of safe food including aquaculture products. A One Health approach is needed to solve this global challenge, but studies that explore the link between AMR from animals, humans and the environment are very scarce. The objectives of this study were to assess the presence of antibiotic-resistant *Escherichia coli* in the rainbow trout farm environment, and to explore the similarity with antibiotic-resistant *E. coli* from farmers and farm employees. *E. coli* was selected for this study because it is an indicator bacteria of fecal pollution from mammals and birds, and an indicator of AMR in the environment.

Materials and Methods

Two farms located in Brittany (NW France) were enrolled in this longitudinal study. Both farms grew rainbow trout (grow-out phase mainly) in continuous flow systems with raceways. Farms A and B were located on different watersheds and represented different risk scenarios with regards to upstream microbial pollution from aquaculture, livestock and humans. Farm A was located downstream from two fish farms and several cattle and swine farms. The closest wastewater treatment plant was 10 km upstream. On the opposite, Farm B reported that no livestock or fish farm was operating upstream. However, a wastewater treatment plant was located closer to farm B (5 km upstream). Farmers reported the use of thrimethoprim-sulfadimethoxine (farm A) and florfenicol (farm B) in the year prior to the beginning of the study.

Two raceways per farm were selected for the monthly monitoring of *E. coli* in water, biofilm and fish. A total of six sampling events took place at each farm, from February to August 2020. At each farm and sampling event, twenty samples per raceway were collected: 10 water samples (100 ml each), one biofilm sample and 9 fishes (feces and internal organs). In total, 240 samples per farm were processed for the isolation of *E. coli*. Farmers and farm employees from both sites were asked to send their fecal samples to the lab for the isolation of *E. coli*. Eight human fecal samples were received in total, five from farm A and three from farm B.

All samples were plated on EMB agar and incubated at 37°C for 48 h. Presumptive *E. coli* colonies were confirmed by MALDI-TOF. Antimicrobial susceptibility was tested by the broth microdilution method against the following antimicrobial agents: enrofloxacin, oxytetracyclin, thrimethoprim-sulfadimethoxine, flumequine, oxolinic acid, colistin and florfenicol. Epidemiological cut-off values were used to classify an isolate as susceptible or resistant, preferentially those determined by the EUCAST. When not available, cut-off values were either inferred from the literature or calculated with the Normalized Resistance Interpretation (NRI) method. The automatic Excel programmes were made available through courtesy of P. Smith, W. Finnegan, and G. Kronvall. Table 1 shows the cut-off values used in this study.

Results

Sixty-seven *Escherichia coli* isolates were obtained during the study period: 42% from farm A and 58% from farm B. In particular, 36 isolates were obtained from water, 28 from fish farmers and three from fish. On farm A, *E. coli* was found in water, fish and fish farmers. On farm B, only water and fish farmers were positive for *E. coli*. This bacterium was not isolated from biofilm samples from any of the farms.

25 out of the 67 *E. coli* isolates did not show phenotypic resistance to any of the antibiotics tested (11 from farm A and 14 from farm B). On the opposite, none of the three isolates from fish (farm A) were pansusceptible. Table 1 shows the percentage of isolates that were resistant to each of the antibiotics tested, and the sample type of origin. The majority of resistant isolates (25 out of 42) showed resistance to only one of the antibiotics tested. Interestingly, all isolates showing resistance to three or more agents (6) were found in water and farmers from site B.

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Table1. Cut-off values used for each antibiotic tested, percentage of resistant isolates and samples of origin

Antibiotic	Cut-off value	% of resistant isolates	Sample type
Enrofloxacin	0.125 mg/l	17.9% (12/67)	water and farmers
Oxytetracycline*	8 mg/l	29.9% (20/67)	water, farmers and fish (sole resistance found in fish gastrointestinal tract)
Thrimethoprim-sulfadimethoxine*	1 mg/l – 19 mg/l	20.9% (14/67)	water and farmers
Flumequine*	16 mg/L	6% (4/67)	water and farmers, only farm B
Colistin	1 mg/l	25.4% (17/67)	water (mostly) and farmers
Florfenicol*	32 ml/l	0% (0/67)	-
Oxolinic acid*	1 mg/l	10.4% (7/67)	water, farmers and fish

*antibiotic authorized for its use in aquaculture in France, only under veterinary prescription

Discussion

In spite of the two enrolled farms being different risk scenarios with regards to upstream pollution from livestock and humans, the overall occurrence of *E. coli* is very similar in both sites. *E. coli* in rainbow trout farms is mainly found in water and farmers, whereas fish are unlikely carriers. The three fish that were positive for this bacterium were in fact diseased animals (furunculosis), thus there may be a link between poor health condition and the colonization of the gastrointestinal tract by *E. coli*. Our results also suggest that biofilms found in freshwater fish farms do not act as a reservoir of *E. coli*.

With regards to AMR, similar phenotypes are found in water and farmers. Resistance to oxytetracycline for example is the phenotype most frequently found in *E. coli* in this study setting, and it is also found in all sample types but biofilm: water, farmers and fish. Oxytetracycline is one of the antibiotics used in aquaculture in France, thus this result is not surprising. However, this finding in *E. coli* from fish farmers may be due to the widely spread resistance against tetracycline in *E. coli* from humans rather than to the use of oxytetracycline in aquaculture. Interestingly, the second most frequent resistance phenotype in this study is resistance to colistin, which is not primarily used in aquaculture in France and is of critical importance for human medicine, according to the WHO. Resistance to colistin was mainly found in *E. coli* from water samples, suggesting they originate from upstream pollution. This antibiotic is mainly used in poultry and pig farms in France, thus we were expecting to find more colistin-resistant *E. coli* in samples from farm A. The fact that farm B is closer to a wastewater treatment plant may explain the similar occurrence of colistin-resistant *E. coli* in both farms.

INTERACTION OF DIET, HOST GENETICS AND GUT MICROBIOTA DURING THE PRODUCTION CYCLE OF GILTHEAD SEA BREAM

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Introduction

The AquaIMPACT H2020 EU project aims to integrate selective breeding and nutrition for producing more sustainable marine fish with a higher phenotypic plasticity to deal with environmental challenges. At this interplay between diet and genetics, gut microbiota emerge as a reliable criterion to assess the selection practices. However, the influence of the host genome on the composition and activity of gut microbiota is still scarcely investigated in gilthead sea bream (Piazzon et al., 2020). Thus, the aim of this study was to unravel the main effects of alternative diet formulations along the production cycle of gilthead sea bream selected for high growth (HG) compared to reference (REF) fish, establishing correlations between intestinal gene expression and gut microbiota abundance.

Methods

HG and REF gilthead sea bream were fed CTRL (15% FM; 6-8% FO) and FUTURE (7.5% FM; 12% poultry sub-products and 3% DHA-rich algae oil) diets during 12 months at the experimental facilities of ECOAQUA, at ULPGC, under natural photoperiod and temperature conditions. Initial (t_0; Nov-19; 10 g body weight; 18 fish), intermediate (Jul-20; t_1; 200 g; 36 fish) and final (Nov-20; t_2; 350 g; 40 fish) sampling points were established to assess the dynamics of anterior intestine (AI) mucosal adherent microbiota composition across the production cycle. As the most prominent differences in gut microbiota among groups were found in t_1, fish of this sampling point were selected for RNA-sequencing. V3-V4 region of the 16S rRNA of each sample was amplified and sequenced by Illumina MiSeq, and taxonomy was assigned using the RDP database. RNA-seq libraries were sequenced by Illumina NovaSeq6000. Good-quality reads were mapped against CSIC gilthead sea bream genome and differential expressed transcripts (DET) were retrieved using DESeq2 (FDR < 0.05). Over-representation analyses of GO and KEGG terms were implemented in goseq and protein-protein association networks were retrieved using STRING (FDR < 0.05). DET expression and OTUs abundances of the equivalent fish of t_1 were correlated using Spearman correlation tests ($P < 0.001$).

Results

Illumina sequencing and taxonomic assignment of the 94 samples allowed the identification of 1,897 OTUs at 97% identity threshold along the gilthead sea bream production cycle. Taking into account the entire population, Chao1 richness index progressively decreased with age, whereas the α-diversity Simpson index was significantly higher in t_1 and t_2 than in t_0. Proteobacteria was the most abundant phylum (64-78%) in all the sampling times, followed by Firmicutes (13-15%), Actinobacteria (6-12%), and Bacteroidetes (~2%). The core microbiota of gilthead sea bream along the production cycle was constituted by 25 OTUs (>1% in at least one of the three sampling times). A validated PLS-DA (Figure 1A) rendered the separation of the three experimental time points. The FUTURE diet induced a shift in the gut microbiota composition of REF fish, validated by PLS-DA, but not in HG fish. The influence of the genetic background was already evidenced at t_0, when discriminant analyses separated HG from REF fish. The magnitude of this separation and the number of discriminant taxa persisted along time, but progressively decreased in t_1 and t_2 in fish were fed CTRL diet. By contrast, HG and REF fish microbiota was not distinguished when fed the FUTURE diet. In t_1, RNA-seq mapped reads were associated to 41,582 AI transcripts, with 1,429 DET (1,218 unique descriptions). Group comparisons displayed 1,057 and 459 DET differentially regulated between HG-CTRL and REF-CTRL fish, and between HG-FUTURE and HG-CTRL, respectively. A total of 1,025 significant associations were established between the abundance of 96 OTUs and the expression of 476 DET. A total of 35 correlations between 20 abundant (>1%) OTUs and 25 DET were stablished (Figure 1B). The 60% of the correlated OTUs were associated to DET related with immune system, with a clear presence of Bacilli linked to anti-inflammatory responses. The microbiota-independent list of DET (953) showed the over-representation of transcripts involved in β-oxidative processes of very long chain fatty acids (up-regulated in HG-CTRL) and smooth muscle contraction (up-regulated in HG-FUTURE).

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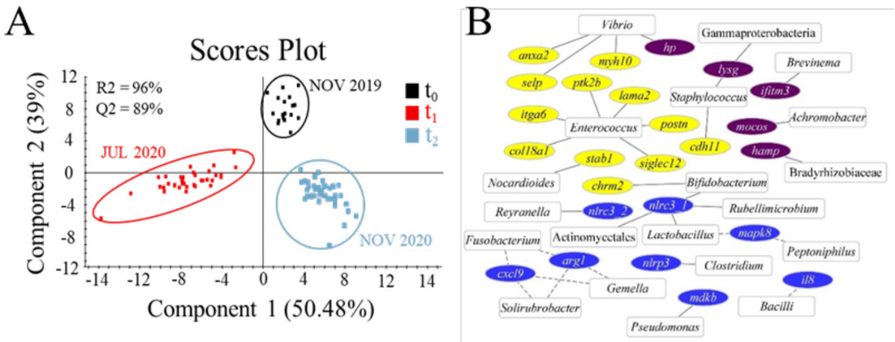

Figure 1. (A) PLS-DA score plots of gut microbiota separating the three sampling points along gilthead sea bream production cycle. (B) Correlation network showing significant correlations (Spearman, $P < 0.001$) between OTUs (white) and DET (yellow, blue, purple) in the anterior intestine.

Conclusions

These results highlight the plasticity of gilthead sea bream gut microbiota along its production cycle, showing the effects of size and season. As in previous studies (Naya-Català et al., 2022), the genetic background strongly influenced the gut microbial composition and the intestinal response of this species, as selected fish exhibited a more plastic microbiota and more changes at the transcriptomic level, when facing dietary changes. Genes related with cell membrane fluency were up-regulated in HG-CTRL fish, whereas FUTURE diet boosted genes involved in intestinal motility in both fish groups. Correlation studies denoted the close link between immune system genes and the intestinal microbiota. Altogether, these results show how diet and genetics can affect the microbiota and the digestive process, and highlight that certain taxa could be interacting with the gilthead sea bream transcriptome.

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REGENERATIVE CELLULOSE FIBERS: POTENTIAL USE OF LYOCELL TEXTILES IN SHELLFISH AQUACULTURE

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Introduction

The purpose of the presented study was to test alternatives for plastic gear in aquaculture application. Microplastics are a major threat to the oceans and their inhabitants, causing increasing problems in all parts of the world. Microplastic contamination and enrichment has been detected in shellfish as well as in several fish meals in different countries.

Therefore, Lyocell (CLY, Lenzing AG) was tested as blue mussel spat collector band. Lyocell origins from cellulose, made of sustainably grown wood, is produced in a closed cycle and is biodegradable (certified with OK biobased certificate, Vincotte certificate). In general, significantly less water is needed for production of Lyocell than for other synthetical as well as natural fibers such as cotton.

Material and Methods

Cellulose fiber bands are tested for their use in blue mussel cultivation in the German Baltic Sea. Therefore, settlement of spat and durability of the cellulose material were tested in field trails within 2022 and compared with commercial Polypropylen (PP) bands. Samples were deployed at May 2nd 2022 before spat fall. Lyocell collector ropes were woven in the STFI and were tested in field for blue mussel spat collection (see Fig. 1a. and b.). Practical work was carried out in Kiel Bay, Baltic Sea in cooperation with the *Kieler Meeresfarm*, Kiel, Germany. Material durability and traction measurement was tested in the Laboratory of the STFI.

The number of mussels per sector of 15 cm was determined and the largest 35 mussels were measured and weighed. The data obtained was compared for statistical variance and significance between the two types of mussel collectors using SPSS.

Results

At sampling date (Aug, 08th 2022) attachment of blue mussel was comparable between Lyocell and PP bands. Mean mussel size was 14,1±0,9 mm and av. 895 individuals per sector for lyocell, and 13,0±2,7 mm with av. 772 mussels per sector for PP. Mussel settling and growth was significantly higher on Lyocell collectors ($p<0.001$).

Discussion

Microplastics are of major concern for the ecosystem and in particular are dangerous in mussel aquaculture, causing disorders of protein metabolism in mussels. Replacement of plastic collectors was tested and initial results indicate suitability of Lyocell textiles for spat collection, as attachment of spat, number of mussels and size of them was significantly higher on Lyocell than on plastic collectors. After three months under water, Lyocell still had enough strength and no ropes or collector bands broke.

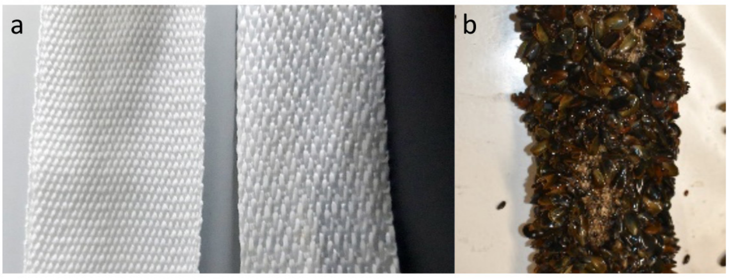

Figure 1a: Lyocell collector band (Lenzing AG) and original sample used, made of polypropylene side by side. 1b: Lyocell band, after three months with on grown mussels.

AN UPSTREAM-DOWNSTREAM EXTERNALITY RELATED TO WATER QUALITY: THE CASE OF THE PORONG RIVER IN INDONESIA

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Introduction:

In this paper, we discuss an upstream-downstream externality related to water quality (level of ammonia) in a river by distinguishing between a non-cooperative and cooperative case. For empirical case, we use the Porong River, which is located in the East Java province of Indonesia. In this river, aquaculture farmers are located downstream while the industrial firms are placed upstream. Using production economic modeling, we find the following interesting results. In the non-cooperative case, we find that the optimal profit by a polyculture is higher than the optimal profit for an industrial firm, and this result is robust to variations in relevant parameter values. Furthermore, by comparing the non-cooperative and cooperative cases, we find that the optimal gain of moving to joint management is reasonably high, and when varying relevant parameter values, this gain remains almost unchanged.

Results:

For the Porong River, we obtain two main results: 1) In the non-cooperative case, the profit for the polyculture farmer is higher than the profit for the industrial firm and, 2) The gain of moving to joint management (or the damage cost of the upstream-downstream externality related to water quality) is reasonably high. Due to uncertainty about the estimated parameter values, we also con-ducted sensitivity analysis by varying each relevant parameter value by +/- 50 % separately. For all parameter variations, we find that the profit is higher for the polyculture farmer than it is for the industrial firm and that the variation in the gain of moving to joint management is very low.

Conclusion:

Finally, in this paper, we have identified the gain of moving to joint management for the Porong River in Indonesia using production economic modelling. We also show that when moving from a non-cooperative case to a coop-erative case, the upstream industrial firm will loss profit while the downstream polyculture farmer will gain profit. An important issue is how to ensure that the cooperative solution is achieved.

CAPTURING AN APHRODISIAC: IMPROVING THE CATCH EFFICIENCY OF OYSTER SPAT (*Crassostrea gigas* & *Ostrea edulis*) IN THE DUTCH SOUTHWESTERN DELTA

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The oyster cultivation sector in the Netherlands has been struggling for several years with a sharp decline in oyster production as a result of variable spawning rates, and mortality in oyster spat (<1 year). The high mortality is partially due to the oyster herpes virus (Ostreid herpesvirus 1; OsHV-1) and predation by the Japanese oyster drill (*Ocinebrellus inornatus*). In order to improve the oyster production volumes in the Netherlands a project was started aimed to increase the catch efficiency of both Pacific oyster spat (*Crassostrea gigas*) and European flat oyster spat (*Ostrea edulis*).

The conventional method of collecting natural oyster spat in the Netherlands is by seeding empty mussel shells on bottom plots. In the Oosterschelde this is mainly done on intertidal plots whereas in Lake Grevelingen this is only possible on subtidal plots.

As a new method, different types of substrates were used in order to collect oyster spat on both intertidal and subtidal plots: loose mussel shells on bottom plots; coupels; and mussel shells in plastic and biodegradable (PLA) nets. Furthermore, in the first year, half of the experimental units were covered with a calcium layer while the other half were not. On intertidal locations, mussel shells were placed in open cages to prevent them from flushing away due to tidal cycles. The coupels and netted mussel shells were mounted on iron A-frames and placed on the bottom in such a way that the substrates were hanging in the water column.

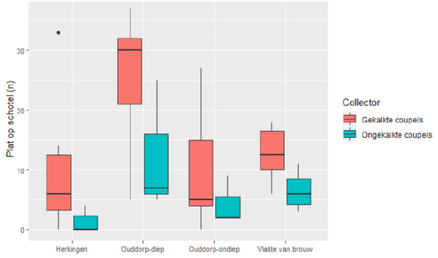
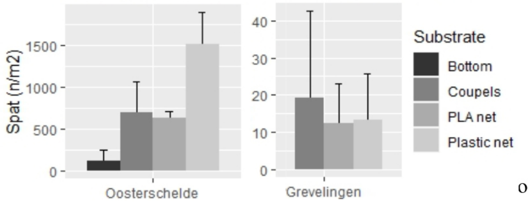


Fig. 1 European flat oyster spat collected in 2020 on coupels with calcium layer (red) and without a calcium layer (blue) on subtidal locations in Lake Grevelingen expressed in number of spat per coupel.



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Grevelingen (b) expressed in number of spat per m^2. Note that the y-axis differ.

To determine the seasonal larval peak, water samples were taken weekly at various locations within the research area and analyzed for the presence of larvae of both oyster species. The substrates were deployed in the field during the larval peak in July, e.g. before the settlement of the spat. Monitoring of the oyster spat took place in November, after the settlement of the spat. The monitoring was carried out by means of destructive sampling, whereby a fixed amount of substrate material from the various test locations was collected and oyster spat was counted.

The first year of experimentations was focused on assessing the best substrates. Only coupels with a calcium layer in Lake Grevelingen resulted in higher catch efficiency of spat (Fig. 1).

The second-year was used to deploy the best substrates on a larger scale. So far, the results of the second year show us that in the Oosterschelde (a), the netted mussel shells seem to collect more spat per surface area compared to coupels. However, for Lake Grevelingen (b) this is the opposite.

Data analysis of the second year of the project is currently ongoing and the results of both years will be discussed during the presentation.

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HANDLING STRESS RELATED MORTALITY OF WHITELEG SHRIMP *Litopenaeus vannamei* IN STANDARD OPEN AND ISOLATED TANKS OF RECIRCULATING AQUACULTURE SYSTEM

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Introduction

Whiteleg shrimp *Litopenaeus vannamei* is the most aquacultured crustacean species and the market demand is growing. In addition to traditional pond aquaculture, new indoor technologies like bio-floc systems or recirculating aquaculture systems (RAS) are being developed, but they may pose additional technological, energetic sustainability, animal welfare challenges. It was noticed, that indoor operation and animal handling procedures in RAS often cause substantial stress for shrimp with different negative consequences, even death. The aim of this study was to evaluate the handling stress-related mortality of whiteleg shrimp in a recirculating aquaculture system by comparing standard open and the new type of completely isolated shrimp tank, developed at Klaipeda University.

Materials and methods

Handling mortality was evaluated for three different experimental cycles of whiteleg shrimp from ~1 to ~25 g individuals, cultivated in RAS, located in the Business Incubator of Klaipeda University. Handling is considered as measurements and sorting procedures for experimental purposes. During or shortly after these procedures, the acute mortality rate of handled animals were monitored. In 2021, more elaborated study on growth performance and mortality was conducted, comparing an innovative, completely isolated shrimp tower tank, and 6 standard open 2.8 m^2 tanks – three as a control and three with different handling treatments. There was no additional stress in the control group other than the necessary unavoidable procedures, while handling stress group had different intensity treatment. Additionaly, stress levels in experimental animals were assessed by the concentration of glucose in the hemolymph.

Results and discussion

The significant acute mortality, caused by standard handling procedures during experimental cultivation, was found for whiteleg shrimp in all three cycles, reaching 20-25 %. However, significantly higher mortality rate suffered handled shrimps grown under complete isolation conditions (41.6±12.6 %). The mortality rate were related to the age (or size) of animals, when the highest procedural mortality rate was recorded during the second-third month or at 5-15 g of individual weight. When evaluating the hemolymph glucose concentration as a marker of stress response, no clear patterns were observed. Although glucose concentration is one of the standard markers of stress in crustaceans, it may not reflect the level of procedural stress in shrimp and the resulting acute mortality in this study.

EFFECTS OF HIGH TEMPERATURE EXPOSURE ON SEX DETERMINATION AND GONADAL TRANSCRIPTOMES AND METHYLOMES IN GENETIC FEMALE (XX) AND MALE (XY) NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Reproduction is an essential biological function through which living beings perpetuate themselves. This biological process consists in the production of new offspring from breeders involving the reproductive system. Sex determination (SD) corresponds to any factor initiating sexual destiny towards one or the other path of sexual differentiation (male or female), while sex differentiation corresponds to the underlying mechanisms responsible for the development of functional female (ovaries) or male (testicles) gonads (Hayes 1998). SD can result either from genetic factors and is defined at fertilization (Genetic Sex Determination, GSD), or from environmental factors and is defined after fertilization (Environmental Sex Determination, ESD) (Piferrer et al., 2012). Between these two extremes, some species have a SD depending on both genetic and environmental factors in different proportions. In these species, SD is referred as GSD + ESD.

Environment, through the perception of various abiotic (temperature, pH, photoperiod) and biotic (population density, social structure) factors, can modify the sexual development leading to phenotypic sexual differentiation in a pathway opposite to that genitically defined at fertilization (Devlin et al. 2002)and the mechanisms involved in primary sex determination are now beginning to be defined. A range of gonadal differentiation types have been described for fish, including gonochoristic species possessing purely ovarian or testicular tissues, as well as hermaphroditic species that can initially mature either as males (protandrous. This lability in sex determination and differentiation mechanisms is widespread among fish.

Effect of temperature on SD was reported for 60 teleost fish species (Ospina-Álvarez and Piferrer 2008). However, the underlying genetic mechanisms are poorly understood. Exposure to high or low temperatures during the thermosensitive period for SD can have different effects on the sex ratio of a population. Generally, the most common sex reversal profile is a masculinization effect induced by temperatures higher than the thermal optimum observed for the species (Ospina-Álvarez and Piferrer 2008).

Nile tilapia, *Oreochromis niloticus*, is a gonochoristic tropical fish species with a high commercial interest. This species has a complex sexual determinism involving several factors (Baroiller et al. 2009). Genetic factors are involved with a homomorphic and male heterogametic sex chromosomes system (XX|XY). The effect of minor genetic factors is also reported and temperature can influence sex determination with masculinization observed at temperatures above 32°C producing XX males (Baroiller et al. 2009).

In this context, the aims of our study was to investigate the molecular mechanisms involved in the masculinization process induced by high temperature in genotypic XX female Nile tilapia, and the effects of high temperatures on genetically male (XY) individuals.

Material and methods

Monosex XX and XY tilapia progenies were exposed to a temperature of 36,5°C from 10 to 30 dpf. Gonad transcriptome was analysed at the beginning (15 dpf) and after (40 dpf) the high temperature exposure. In parallel, we characterized the gonad methylome to evaluate the role of epigenetic control on the differentially expressed target genes. Sex ratio of the different progenies was analysed at 90 dpf.

Results and discussion

High temperature treatment induced different effects on sex ratio according to genotypes. In XX progenies, masculinization rate ranged from 20 to almost 100%.

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Surprisingly, an opposite effect was observed in XY genotype, with feminization of some progenies. Sex reversal incidence and rate were lower compared to XX masculinization with only 50% of sensitive progenies and reversal rate ranging from 6 to 52%. To our knowledge, this is the first report of such a SD thermosensitivity profile in a vertebrate species.

Our study introduces for the first time a correlation between gene expression and observed inversion rate (masculinization or feminization). Differential expression analysis allowed to identify more specifically genes directly involved in high temperature sex reversal process in *Oreochromis niloticus*. Feminization and masculinization have different characteristics in genes expression pattern. Indeed, during masculinization we observe an induction of known male pathways coupled with a repression of known female pathways. Conversely, during feminization we observe an induction of the known female pathways but the male pathways seem to persist.

We identified *foxl2* gene, known to be widely involved in the female determination and differentiation pathway in vertebrates and tilapia. In our study, *foxl2* presents a particularly interesting expression profile. Indeed, as we have shown, during masculinization it presents an onset of repression at 15 dpf, which is a function of the rate of inversion observed, and at 40 dpf is clearly under-expressed. In a symmetrically opposite way, we identify an induction of its expression already at 15 dpf during feminization and also at 40 dpf. Among the other sex determinism genes identified, we interestingly observe a constant induction of the *gsdf* gene following the heat treatment regardless of the stage and the genotype.

Our study highlighted difference in expression of genes related to metabolism. Indeed, we have demonstrated a repression of the expression of pathways involved in metabolism during masculinization and an induction of these during feminization.

Differences in methylation patterns according to masculinization or feminization were also highlighted. The study of the methylation difference also shows methylation modifications at the level of genes involved in metabolism with opposite profiles between masculinization and feminization.

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PIKEPERCH CULTURE IN AQUAPONICS : PRODUCTIVITY AND WELFARE IN MONO- AND POLYCULTURE

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Introduction

Interest is growing for aquaponics as a way to reduce environmental negative impact of running aquaculture and hydroponics systems separately. The PERCIPONIE project (Interreg GR) aims to develop percid fish culture in aquaponic systems. Intensive pikeperch (*Sander lucioperca*) aquaculture is still in its infancy. Moreover, this specie is considered as a good prospect for the European market due to its fast growth rate and economical expectation. Those characteristics made it a perfect candidate for the « Perciponie » project. The main risk of an aquaponic system regarding water quality and fish welfare is the accumulation of high concentration of nutrients (particularly nitrate) into the system in order to support the best growth of the plants.

The aim of the work was to assess the welfare and production performances (growth and survival) of pikeperch in aquaponic water quality conditions.

The study was performed in two steps : first we experimentally test the effect of different nitrate concentrations on growth performances, survival rate and welfare of pikeperch. Secondly, we evaluate these parameters in a coupled aquaponics pilot system in pikeperch monoculture and pikeperch/sturgeon polyculture

Material and Methods

First, we exposed pikeperch to increasing concentrations of nitrates (354 mg/L to 2655 mg/L NO_3^-) and measured growth indicators such as weight gain, specific growth rate and feed conversion ratio. 20 juveniles (194,3±28.3 g) were randomly distributed in 250l aquarium with an individual filtration system. Nitrate solutions were added to the water with a peristaltic pump to maintain nitrate concentrations at 6 different experimental values. Fish were fed at 1% of biomass/day. Fish growth and survival were measured and welfare was evaluated at the end of the experimental period (60 days) on 5 fish by measuring plasma cortisol, brain serotonin and dopamine levels by ELISA assays.

Secondly, pikeperch was reared in real aquaponic conditions to assess the productivity of the system. The aquaponics facility surface: 100 m², total volume: 18 m³) allows to assess the productivity in real production conditions. Pikeperch were reared at 23°C.Average pH and oxygen levels were 6,9 and 8,2 mg/L respectively. Stoking densities varied between 30 to 88 kg/m³ depending on the size of fish.

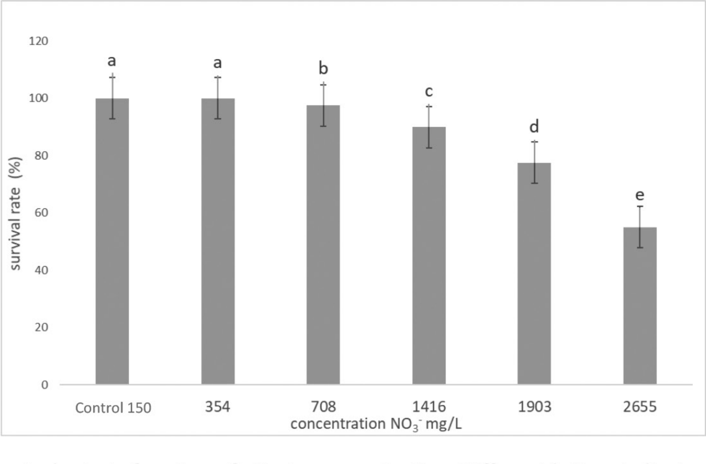

Figure 1. Survival rate in function of nitrate concentration. Different letters indicate a statistical différence (p < 0,05).

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Initially, the aquaponic pilot was exclusively stocked with pikeperch and the vegetal production consisted solely in lettuce. In a second time, a polyculture approach was tested withpikeperch and sturgeon (*Acipenser ruthenus*). Fish and vegetable productivity was assessed on a 74-day experimental period. Pikeperch welfare was evaluated by measuring cortisol, serotonin and dopamine levels.

Results and discussion

Pikeperch exposed to increasing nitrate concentrations showed a decrease in survival rate above 354 mg/L NO_3^- (Figure 1). This indicates that the toxicity threshold value is between 354 mg/L and 708 mg/L. Survival rate was close to 100% and similar between 150 mg/L, 354 mg/L NO_3^- and the control. Growth indicators tend to decrease with the increase in nitrate concentrations. However, only the highest concentration (2655 mg/L NO_3^-) led to a statistical reduction in growth compared to control. Plasma cortisol levels were similar in all groups. Dopamine and serotonin analysis are in progress..

First productivity results of pikeperch monoculture in real aquaponic conditions were assessed during 74 days. Mean nitrate concentration in the system during the experimental period was 321 mg/L, which is under the toxicity threshold previously assessed for growth and survival. Pikeperch annual production reached 160 kg with a mortality rate of 7,8%. Lettuce mean weight reached 267,2 g ± 54,6 g. Seven weeks are needed to fully grow a lettuce, 1 week of germination on rockwool followed by 6 weeks of deep water culture on hydroponics rafts. Based on those data, we estimated an annual production of 1282 kg/an. Welfare analysis and comparison with pikeperch-sturgeon polyculture are still in progress.

Final results will be very helpful to guarantee pikeperch welfare and support the development of pikeperch culture in aquaponics.

INVESTIGATION OF HIGH-PRESSURE PROCESSING ON THE EXTRACTION OF FREE AMINO ACIDS FROM DUCKWEED (*Lemna minor*)

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Introduction

Over the years, there has been a strong interest in the downstream processing of aquatic plants to extract proteins, as it is instrumental in fulfilling the nutritional demand of the emerging population. Duckweed (*Lemna minor*) of the *Lemnaceae* family is widely known as the smallest, fastest-growing flowering plant. Due to rich essential (39.20%), non-essential (53.64%), and non-proteinogenic (7.13%) amino acids, it is considered a "water lentils" for human consumption (Chakrabarti et al., 2018; de Beukelaar et al., 2019; Pagliuso et al., 2022). Several conventional extraction strategies are employed to recover amino acids from duckweed; however, these methods are exorbitantly expensive and time-consuming. Therefore, in the present study, high-pressure processing (HPP) and conventional assisted extraction (CAE) were employed to recover flavour-active free amino acids (FAAs) from Duckweed, and the objective was to investigate the effects of HPP and CAE on the aqueous extraction of FAAs from its dry biomass.

Material and methods

The lyophilised duckweed powder was used for this work. Initially, seven extraction strategies (i.e., HPP (n=5), and CAE (n=2) were investigated. Based on the yield of total soluble solids post-HPP and CAE treatment, samples were subjected to FAA profiling, which was carried out using a UHPLC-FLD method. About sixteen FAAs were found in the extracts, including all nine essential amino acids. Comparison between the matrices of FAAs composition was also carried out using principal component analysis (PCA) on the results of the extracts.

Results

Results showed that the primary amino acids extracted are aspartic, glutamic, threonine, alanine, valine, leucine, and arginine. Overall, the highest concentration of FAAs was found in samples treated with 200 and 400 MPa for 4 min treatment time (Figure 1). This study demonstrated the highest extraction of umami flavor (aspartic and glutamic acids) using these novel extraction technologies from *Lemna minor*. Additionally, HPP treatment reflected significantly higher extraction of FAAs compared to conventional treatment.

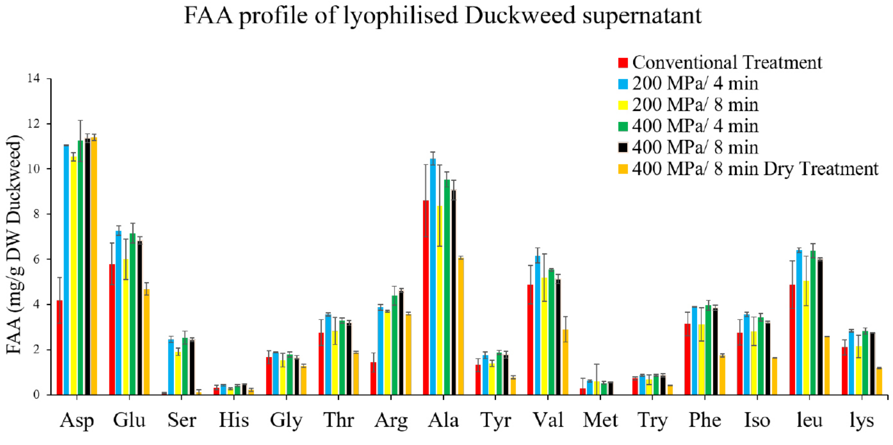
FAA profile of lyophilised Duckweed supernatant

Figure 1. Illustrates FAA profile of Duckweed extracts treated with HPP and CAE

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CAN THE IMMUNE SYSTEM OF SHRIMPS BE TRAINED TO FIGHT VIBRIOSIS ?

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Introduction

Vibriosis caused by pathogenic *Vibrio* species is one of the major constraining factors for the sustainable production of farmed shrimps. The immune system of shrimps consists of only the innate immune system, which is believed to lack adaptive features i.e., specificity and long-lasting memory. However, studies carried out in the past on invertebrates (e.g., insects, honey bees, oysters) suggested that their innate immune system could be trained to mount enhanced and long-lasting protective immune responses against both related and unrelated pathogens (Salminen & Vale 2020; Kangale et al. 2021). This capacity of innate immunity to respond adaptively to secondary challenges represents a memory-like characteristic of innate immunity (Norouzitallab et al. 2019). The emerging view that innate immunity could be trained for having long lasting memory has generated immense interest in the shrimp research community to carry out extensive research focusing on this area. We conducted a series of transgenerational studies using the brine shrimp *Artemia* as a model for shrimps to study innate immune memory and the transfer of this trained immunity from parents to offspring. We also focused on possible mechanisms associated with it. The specific aim of carrying out such studies is to develop a robust, cost-effective, and more sustainable disease-control strategy. We used the brine shrimp *Artemia* as a model for farmed shrimps to study the impact of primary exposure of the parental generation to *Vibrio* spp. on the resistance of the progenies against homologous or heterologous challenge. The results suggested that training the immune system, induced by exposure of the parental population of *Artemia* at their early life stages to variouse species of *Vibrio* (relevant to aquaculture species) caused a significant increase in the resistance of the three successive generation progenies (of which none was exposed to the parental stressor) against subsequent challenge with a homologous or heterologous *Vibrio* strain. Protection in the infected offspring was more pronounced when they were infected with the homologous *Vibrio* strain. Interestingly, this transgenerational inheritance of acquired vibrio-resistant phenotype was associated with elevated levels of the signaling molecules such as heat shock protein 70 (*hsp70*) and high mobility group box 1 (*hmgb1*), alteration in the expression of selected innate immunity-related genes, and modifications of the selected epigenetics marks, such as global acetylation level of H4 and tri-methylation level of H3K4, in the progenies whose ancestors were trained by *Vibrio* exposure (Norouzitallab et al. 2016; Roy et al., 2022). These pioneering studies were the first to provide evidence for the occurrence of innate immune memmory in a crustacean species, and to provide some insight into the mechanisms involved. Building upon these findings, recently, several other studies were conducted both by our group as well as others to examine in a comprehensive manner the occurrence of immune memory in shrimps and other farmed crustaceans, and the molecular mechanisms behind such possible phenomena. In this review, we aim to bring this information together to address the question whether the shrimp's innate immunity could be trained to protect them against pathogenic vibrios. We believe that the knowledge generated from these types of research studies will revolutionize our understanding of the defense systems not only in farmed shrimps but also in commercially important farmed invertebrates (like crayfish, lobsters, mussels, honeybees). The findings of this study can be useful for the development of prophylactics, such as vaccines relevant to these species.

Acknowledgments

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EFFECTS OF FASTING AND WEIGHT GAIN ON PROXIMATE COMPOSITION AND FATTY ACID PROFILES OF EUROPEAN SEABASS *Dicentrarchus labrax*

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Introduction

The effect of re-feeding after fasting on the compensatory growth of aquatic animals has always been an interesting topic in aquaculture and fish nutritional physiology. Feed deprivation and re-feeding can significantly affect proximate composition of fish (Qiu *et al*., 2017). The primary goal of the present study was to investigate how different fish sizes as an individual variable (small vs big) can affect the growth performance when European seabass *Dicentrarchus labrax* individuals are subjected to feed deprivation. Secondary goal was to examine the effects of a specific feeding strategy on proximate and fatty acid composition at different sizes of *D. labrax*, which is a fish species of great economic importance in the Mediterranean industry.

Materials and Methods

The experiment was carried out in a Recirculating Aquaculture System (RAS) at the CARUS research facility of Wageningen University and Research in the Netherlands. Fish with an initial weight of 87.5±16.2g were randomly allocated in 6 tanks (90 fish/tank), supplied with artificial seawater. Fish were distributed into two groups and each treatment was tested in triplicate. The control group was fed daily (twice) *ad libitum* with commercial pellets (Altech Coppens, The Netherlands) whereas the second group was fed for 8 days and deprived for 2 days. This sequence was replicated for 40 days. Throughout the experimental procedure water temperature and pH were maintained at 17.2±2.4°C and at 7.37±0.23, respectively. At the end of the experiment, fish from each treatment (fed and fasted) were classified as fast (FG) and slow growers (SG) according to their final weight and 9 fish from each group were randomly captured and sampled. Crude protein content was analyzed using the Kjeldahl method. Lipid content was measured using the Soxhlet method and ash content was obtained after combustion at 600 °C for 5 h. Energy content was determined using an IKA oxygen bomb calorimeter (C5000, IKA Werke, Staufen, Germany). To determine fatty acids' profiles Folch extraction method was used, followed by esterification and separation using a capillary column in an Agilent GC (6890). One-way analysis of variance (ANOVA) followed by Tukey post-hoc test, was used for each treatment. Main effects and interactions among experimental groups were identified using two-way ANOVA. Mean differences were considered significant at $P < 0.05$.

Results

All fish adapted well to the feeding regimes, and no mortalities were recorded throughout the experimental period. At the end of the experiment, both fast growing (FG) and slow growing (SG) groups of fed and fasted fish increased their weights. Within the FG and SG groups, the experimental feeding treatment did not significantly affect final body weight. Furthermore, the intraperitoneal fat ratio (IFR) for both FG and SG groups, was not affected by dietary status ($P > 0.05$) and the highest IFR values were observed for the fasted ones. In addition, significant differences ($P < 0.05$) were observed among the groups for moisture, dry matter, proteins, ash and gross energy, but no significant changes were found in lipid content ($P > 0.05$). Fed experimental group exhibited significantly higher EPA (20:5n-3) and DHA (22:6n-3) than their fasted counterparts. Except for DHA, no interaction between nutritional status and growth was observed. The lipids of SG contained significantly lower proportions of 18:1n-9, 18:2n-6, Σn-6 and 18:3n-3 and higher proportions of 14:0, 18:4n-3, 20:1n-9, 22:1n-11, EPA and DHA, compared to the FG.

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Discussion

The proximate and fatty composition of different sized seabass individuals were evaluated. In the present study, the final weight between the FG and SG groups were not significantly affected by the short-term food deprivation period. Furthermore, this period did not have a substantial effect on IFR. On the contrary, Caruso *et al*. (2011) have mentioned a decline in growth under a short-term starvation period on European seabass and blackspot seabream. The need for the development of a suitable feeding strategy during production in aquaculture, is urgently required. Therefore, the specific feeding schedule concerning starvation and re-feeding cycles could be a likely feed management choice for the culture of this species. Also, no considerable changes occurred in carcass lipid content, between fed and fasted groups, nevertheless it was measured higher protein content in SG compared with the FG, only for the fed fish. On the contrary Yılmaz & Eroldogan (2011) measured higher protein content in fish from the control group. Bigger fish usually have higher lipid and protein status, compared with the smaller ones, as they accumulate more energy stores. As a result, larger fish usually evolve better adapted mechanisms to control food deprivation period and are less sensitive to mortality. Different feeding regimes may affect fatty acid contents (Ambrosio *et al*., 2008). In this study, high levels of saturates, especially 14:0 and 16:0 were observed in fed SG compared to the fasted SG. It is evident that most of the aforementioned fatty acids were affected not only by the short-term food restriction but also by the fish size. Such feeding protocol could be helpful for aquaculture through controlling survival and growth and optimizing the feeding costs.

Acknowledgements

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WATER CIRCULATION PATTERNS' IMPLICATIONS ON SUITABLE SITES FOR CAGE AQUACULTURE IN LAKE VICTORIA

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Introduction

Fish farming in cages in Lake Victoria has been rapidly increasing following the decline of catch rates of wild stocks. However, of recent, massive fish kills have been witnessed in some cage sites. Preliminary studies have attributed the fish mortalities to localized upwelling events that lead to depletion of dissolved oxygen due to release of decomposing matter into the water column. We developed ROMS model to be used to predict such events and ultimately play a critical role in cage culture site selection.

Methodology

We modelled these dynamics using a Regional Oceanographic Model System (ROMS) as from January 1st, 2000. Using the bulk flux technique, the model is based on real bathymetry, river runoff, and atmospheric forcing data.

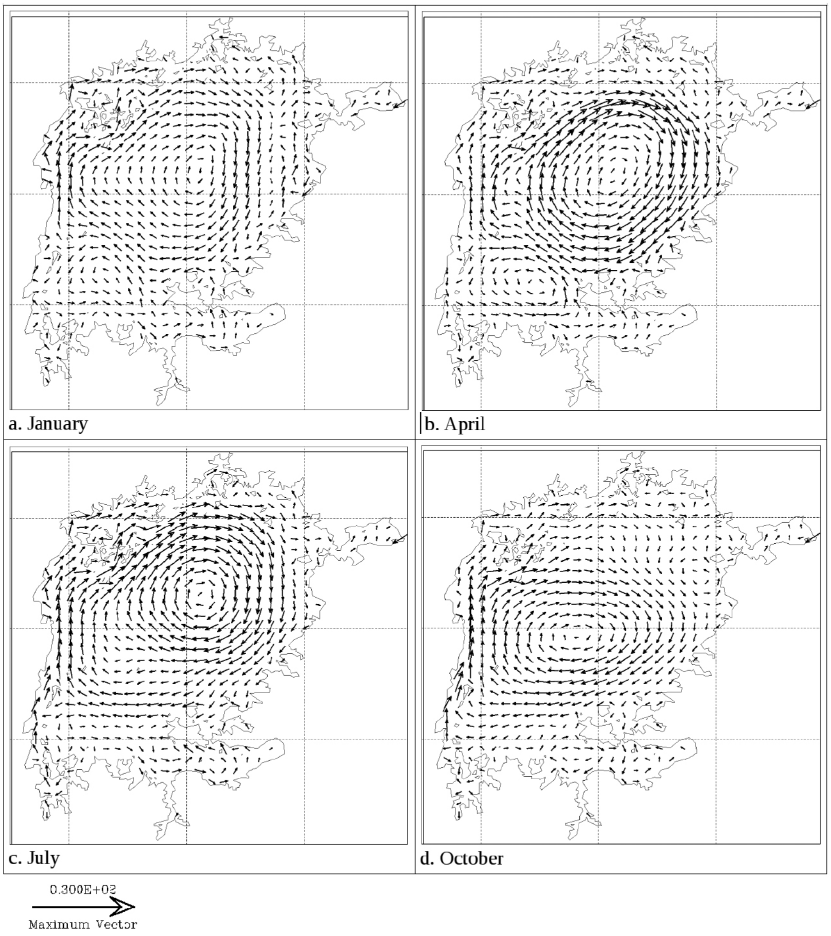
a. January
b. April
c. July
d. October

FIGURE 1 Modelled vertically averaged currents (vectors) in Lake Victoria in different seasons of the year.

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Results

Annual cycles of thermo-stratification (September–May) and mixing (June–August) are observed in the water column, according to simulations. Surface water currents have several forms, ranging from a lake-wide northward flow to gyres of various sizes and numbers. Upwelling and downwelling zones arise as a result of an underflow. The lake's center and western inshore waters have the highest current velocities, implying that water circulation is better there. However, water exchange between the major gulfs (especially Nyanza) and the open lake is minimal, which could explain the water quality discrepancies found in those areas.

The findings of this study add to our knowledge of the physical processes (temperature and currents) that influence diel, seasonal, and annual variations in stratification, vertical mixing, inshore—offshore exchanges, and nutrient fluxes, all of which have an impact on biotic distribution and trophic structure. Information from this study on upwelling areas/timing and vertical mixing, for example, will help anticipate high primary production and, eventually, fisheries productivity in Lake Victoria, as well as optimal cage culture sites.

ATLANTIC INTEGRATED MULTI-TROPHIC AQUACULTURE – THE ASTRAL PROJECT

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Introduction

ASTRAL is a European Union Horizon 2020 collaborative project that focuses on integrated multi-trophic aquaculture (IMTA). The aims of the project include defining, supporting, and promoting sustainable IMTA production across the Atlantic.

Integrated Multi-Trophic Aquaculture (IMTA) is developing as an innovative option for further enhancing the sustainability of aquaculture. Aquaculture faces many challenges in the future due to emerging threats such as the growing risk of harmful algae blooms (HABs) and disease linked with climate change; production and infrastructure losses from extreme events; fluctuations in water temperature and oxygen levels; ocean acidification, and increased demand for resources (Lafferty, 2015). By culturing a combination of lower trophic and higher trophic species together, the co-products of one crop (fed animals) are converted into fertilizer, food and energy for the other crops (extractive seaweeds and animals). This IMTA practice is shown to increase circularity and reduce waste from fed species and lower the nutrient load in the water (FAO, 2018). ASTRAL (EU H2020) focuses on IMTA production in the Atlantic region to address these challenges. 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). The aim of ASTRAL is to boost IMTA with the implementation of innovative and resilient production, while assessing environmental stressors and pollutants occurrences with new technology developments.

Methodology

ASTRAL is engaged with five IMTA research/production sites in the Atlantic basin to assess production methods, management systems, biosecurity, food safety and profitability, these are known as IMTA labs. ASTRAL is assessing IMTA circularity of each of the IMTA lab systems to allow the development of a zero-waste strategies. The IMTA labs are assessing the presence of microplastics and microbial communities to inform biosecurity and health measures for the chosen IMTA systems. Life cycle assessments (LCA) are comparing the environmental impacts of IMTA to those of traditional aquaculture. Affordable sensors and new technologies are being developed to enhance monitoring capabilities for the aquaculture industry.

IMTA lab Brazil is a biofloc system. During intensive shrimp production without water renewal, there is an accumulation of nutrients excretion/feed degradation and organic matter (biofloc) that are removed mechanically and biologically. The integration of IMTA species consuming biofloc (tilapia and oysters) and dissolved nutrients (seaweeds and halophytes) has been investigated as an alternative to monoculture to maintain the water quality in the closed production system.

IMTA lab South Africa incorporates a commercial farm (Buffeljags Abalone) that integrates the cultivation of abalone, in land-based raceway tanks, 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 in the system and *Ulva* is used as a supplementary feed for the abalone. IMTA lab SA is also developing an IMTA land-based system for other high-value aquaculture species - sea urchins.

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IMTA lab Scotland operates an IMTA open-water system to optimise cultivation techniques of macroalgae and shellfish and demonstrate an improved economic case for the co-cultivation of kelps and native oysters. This lab is undertaking environmental monitoring to improve the cultivation system performance (yield and composition, stocking density and biosecurity). The development of new cultivation systems is exploring options to minimise cultivation wastes through improved system design and reducing, reusing, and recycling polymer-based cultivation materials.

IMTA lab Ireland is developing and validating cost-effective IMTA processes in an open-water system. This IMTA system is exploring the feasibility of the cultivation of Atlantic salmon, lumpfish, European lobster, native oysters, scallops, seaweeds and spiny sea urchins. Production technologies are being assessed and optimised to enhance profitability and to mitigate environmental impact. This lab seeks to establish best practice for the cultivation of these species by monitoring and assessing animal welfare, biosecurity and health management.

Prospective IMTA lab Argentina is gaining knowledge from the other IMTA labs. Feasibility studies are being carried out to assess local species (fish, crustacean, mollusc and echinoderm) and to identify appropriate sites within the Beagle Channel to facilitate IMTA development.

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 lessening of waste.

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USING SERIOUS GAMES TO GAIN INSIGHT INTO THE KEY CHALLENGES PERTAINING TO REGULATION OF NEW AQUACULTURE PRODUCTION SYSTEMS IN NORWAY

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Introduction

The World Bank said already in 2013 that "*Feeding an expected global population of 9 billion by 2050 is a daunting challenge that is engaging researchers, technical experts, and leaders the world over*"(World Bank 2013). The impressive growth of the aquaculture industry in the past decades has resulted in more food being produced, keeping the prices of fish down and making it more accessible to consumers around the world – giving us hope that the daunting challenge in fact is possible to overcome.

Globally, Norway is a leading producer of Atlantic Salmon. During the last 10 years, the Norwegian aquaculture industry has experienced an incredible development, with a total revenue growth of more than 200%. Despite the growing demand of salmon, the growth in production volume is currently at a halt due to the environmental challenges faced by the industry. Challenges include salmon lice and escapees, (Thorvaldsen, Holmen et al. 2015, Misund 2019). These challenges have resulted in negative media attention and decreasing social acceptance for the industry (Tiller, Brekken et al. 2012), resulting in a lack of growth of the industry. Consequently, the industry needs to be innovative and improve its sustainability and reputation. Current challenges and opportunities pertaining to regulations of the production systems needs to be identified and assessed to enable innovation and sustainable growth of the industry. And who doesn't love a good board game. For the purposes of this article, Serious Games are defined as games with a purpose beyond that of purely entertainment and enjoyment (though we love that too). Serious games have a long and rich past, having been used in military and educational training for decades. Serious games have increasingly been used to raise awareness about environmental issues and sustainability (Stenros 2017) – but not aquaculture directly in Norway. In this study, we chose to use serious games as a method to collect data about challenges and opportunities related to regulating the different aquaculture production systems in Norway, to gain input and suggestions for sustainable development of the industry and while also allowing us to increase ocean literary among future generations.

Methods

In this study, Serious Games served a dual purpose as a method for harvesting data related to stakeholders' perceptions of the challenges and opportunities, and for increasing societal understanding and awareness about them. Set in the context of the United Nations Sustainable Development Goals (SDGs) and the United Nations decade of ocean science, we developed "The serious(ly) fishy game". The game is developed based on discussions and information gathered in workshops with stakeholders and in collaboration with representatives from the aquaculture industry. The game, which is contextually placed in Norway, was played in three different sessions with experts from the industry as well as with high school students in Norway. To contribute to the sustainable development of the industry, the aim was to collect qualitative expert data in an informal gaming setting on the challenges and opportunities pertaining to regulating new production systems in the Norwegian aquaculture industry. This would be a more engaging method for finding new solutions that we may not have thought of before.

The game starts out with the participants getting general information about the SDGs and being forced to pick three out of four SDGs given to them and rank them according to their perception of their importance. The reason behind limiting the numbers of SDGs for the game was to get a more focused discussion and to save time while playing the game. After having ranked the SDGs, the participants were then presented with information about the aquaculture industry (e.g. export value and challenges related to regulations. Based on this new information, the participants are asked to discuss whether they wanted to change the prioritized order of the SDGs.

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Following the SDGs section, the game had five phases. The first four were directly related to the four specific production systems at hand: land-based, floating closed, floating semi-closed and open ocean aquaculture systems. The participants were presented information about the systems, such as its history, benefits and challenges. Then the participants were given a scenario related to each system that they must first discuss, and then rate the social, economic, and environmental impacts of. After having rated the consequences, the participants are given three countermeasures for each scenario that they had to choose from and rank in terms of social, economic and environmental consequences. The countermeasures had different focus where A was social, B more economic and C more environmental focused. This is something participants were not made aware of until the wrap-up session at the end of the game. The fifth phase contained scenarios that addressed challenges faced by all the production systems. This included sustainable regulations, the use of land produced food for the fish and security threats. The scenarios presented throughout the game were meant to provoke discussions, but at the same time they had to be realistic enough to create a real discussion. The scenarios and the countermeasures were created based on the discussions in the stakeholder workshops. They were further tested, adapted and validated by industry representatives.

The game finish up with a ‘wrap-up’ section where the participants were asked to discuss the decisions they made throughout the game and discuss whether they had focused more on social, economic or environmental impacts when choosing countermeasures. Further, they are asked to discuss how the decisions they made relate to how they prioritized the SDGs in the beginning of the game.

Results

Results from the first round of playing the game shows that the expert participants representing both research and industry see both challenges and opportunities related to the different production systems used in the Norwegian aquaculture industry and that these varies based on what sector they are representing (research or industry) and their age. In this first round of playing the game there was three groups. Two groups ranked the SDGs the same, having SDG 14 as the most important, SDG 12 as the second most important and SDG 8 as the number three. Thereby choosing to get rid of SDG 9. The final group on the other hand ranked SDG 9 as the one with the highest priority and choosing to get rid of SDG 12. Another interesting result was that the two groups that ranked SDG 14 as the most important one, did not choose any of the countermeasures most focused on the environment, but rather countermeasures with an economic or social focus.

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PROJECT RESULTS FROM NETCLEAN 24/7 – PERMANENTLY RESIDENT ROBOT FOR AUTONOMOUS NET CLEANING AND INSPECTION

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Introduction

Biofouling is the growth of organisms such as algae, mussels and hydroids on submerged marine structures. In the sea-based aquaculture industry, biofouling is a challenge as it causes reduced oxygen flow through the fish cages, net deformation, and increased wear and tear on the nets and mooring systems due to occlusion of the nets. Underwater robots utilizing pressure cleaners are often employed to remove the biofouling. This poses a risk to fish health, as harmful cleaning waste is released into the water. An alternative to pressure cleaning is to use brush-based grooming robots that gently disturb the net at regular intervals and hence prevent the biofouling from establishing [1]. Net inspection and monitoring of the environment is also of importance to the fish farmers. The project Netclean 24/7 (RCN 296392) has for the last 3 years developed the autonomous underwater robot 'Remora' for net cleaning and monitoring. This work presents the results of the Netclean 24/7 project and includes an analysis of the robots' functions, modelling of the robot dynamics, autonomous control functions, and a docking station design for permanently resident aquaculture robots.

Materials and methods

Before the development of the robot and its autonomous functions could begin, a thorough analysis of the requirements of the robot was performed. The SEATONOMOY method [2] was used for this purpose. This iterative method aims to define all the required operations the robot must be able to perform, then breaks these operations down into smaller sub-tasks. Once completed, the SEATONOMY method provides the designers with a comprehensive overview of the requirements for all software and hardware necessary to complete the robot [1].

Developing autonomous functions for a robotic system is deemed easier if one has a mathematical model of the robots' dynamics, and the environment it operates in. As such, a model was developed for the Remora robot moving on a dynamically changing fish cage [3].

The autonomous functions of the robot were developed through a series of simulation experiments utilizing the mentioned model. The robot required algorithms that could ensure path following and obstacle avoidance. Based on the investigation of several such algorithms [4, 5], the Elastic Band Method (EBM) was chosen. This method conceptualizes the robot's path as a rubber band between a start point and an end point. If an obstacle appears, the path wraps around the obstacle, much like an elastic rubber band would do in the same situation. To ensure that the robot maintains a safe distance to obstacles, a series of bubbles are placed along the path. The bubbles radii are chosen such that the inside of a bubble is considered a collision free area.

To realize fully autonomous and resident robotic systems in aquaculture a subsea docking station suited for aquaculture sites is required. An extensive study on existing subsea docking stations for autonomous underwater vehicles (AUVs), as well as a concept design for aquaculture use, were performed as part of the Netclean 24/7 project [6].

Results

The results of the Netclean 24/7 project include an extensive analysis of the requirements and specifications for an autonomous, resident biofouling prevention robot. Further, a mathematical model has been developed to describe the dynamics of crawling robots. Advanced motion planning and obstacle avoidance algorithms has been developed and extensively tested, both in simulations [7], and laboratory experiments. A concept for a subsea docking station for aquaculture robots has been designed based on identified requirements from subsea docking stations used in other domains. Figure 1 shows a simulation of the motion planning and obstacle avoidance utilizing the mathematical model. The model captures the interactions between the robot, the net structure, and the ocean currents, while the motion planner is able to re-calculate the path to avoid obstacles. Figure 2 shows the results from the laboratory trials. The robot was able to avoid both static and dynamic obstacles in a controlled environment. Figure 3 shows the subsea docking station concept for residential aquaculture robots. The concept is based on wireless charging and data transfer.

Conclusion and future work

The Netclean 24/7 project consists of project owner Remora Robotics and partners SINTEF Ocean, Nordlaks AS, Xylem Aanderaa AS, and NTNU. The Remora robot is currently operational at several active fish farming locations in Norway and field trials implementing the results of the Netclean 24/7 project are planned in 2022.

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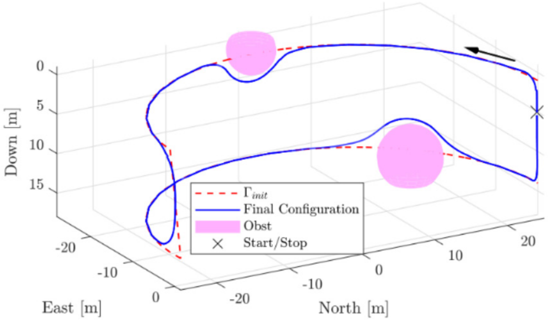

Figure 1: A simulation of the Elastic Band method.

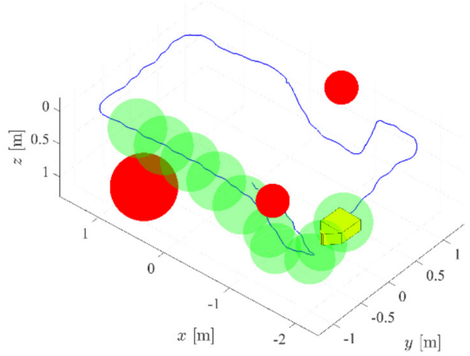

Figure 2: Laboratory trials using the Elastic Band method.

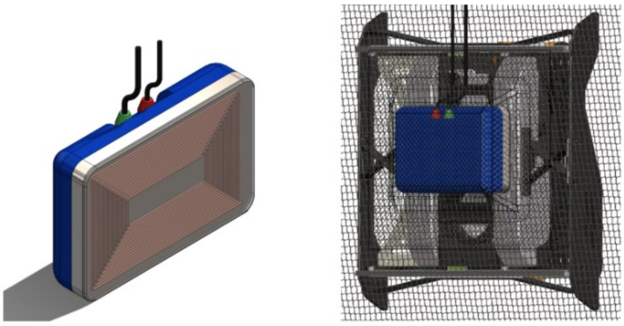

Figure 3: Subsea docking station concept for aquaculture.

COMPARISON BETWEEN PEDIGREE AND GENOMIC PREDICTIONS USING THE MEDFISH SNP-ARRAY AND SELECTED LOW-DENSITY SNP PANELS FOR BODY WEIGHT IN EUROPEAN SEABASS

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Introduction

Genomic selection can improve the selection accuracy and reduce the generation interval. However, pedigree selection has been utilized for growth and body weight in commercial aquaculture breeding programs because of the increased cost of genotyping. Thus, in the present study an effort was made to develop a reduced, low-cost SNP panel to be utilized for genomic selection purposes for production traits (i.e., growth, body weight) and disease/parasite resistance (i.e., parasite count). The aim of the study is to compare the pedigree and genomic predictions using the 30K MedFISH array (Peñaloza et al., 2020) and selected low-density SNP panels for body weight in European seabass (*Dicentrarchus labrax*).

Materials and Methods

For this purpose, a selected sub-sample of 985 fish, from the total of 1,576 fish, infested with *Lernanthropus kroyeri*, was genotyped using the MedFISH array. Two GWAS were performed, one for growth in the sea cage and the other one for the final weight at the end of the experimental period (Oikonomou et al. 2022a, in press) and from these the significantly associated SNPs with the trait of interest (p-value<0.05) were selected. Further, a multitrait GWAS using different growth at different periods in the sea cage was performed and significantly associated SNPs (p-value<0.01) were selected. Based on the above criteria, 1,715 SNPs related to growth were finally selected.

Furthermore, a GWAS was performed for resistance to *Lernanthropus kroyeri* (parasite count, PC) (Oikonomou et al. 2022a, in press). Two low-density panels (SNP–panel 1 and SNP–panel 2) were constructed using two selected groups of SNPs as criteria, according to their p-value from the GWAS (p-value<0.01 and <0.05, respectively). Then, 245 and 1,192 SNPs associated to parasite resistance (GWAS for parasite resistance), were included in the SNP-panel 1 and SNP-panel 2, respectively. *T*hose SNP panels were enriched with the 1,715 SNPs related to growth. Thus, two reduced density SNP – panels, which included 1,960 SNPs (named as SNP-panel 1) and 2,907 SNPs (named as SNP – panel 2) were constructed.

Estimated Breeding Values (EBVs) for the final body weight were calculated using Best Linear Unbiased Prediction (BLUP) and Genomic Estimated Breeding Values (GEBVs) for the final body weight were assessed using Genomic-BLUP (GBLUP) for the three SNP panels (30K MedFISH array, SNP–panel 1 & 2), using BLUPF90. Each time, 20% of the population was selected randomly and its phenotypes were masked (216 fish), thus, the breeding values (EBVs or GEBVs) were estimated using the information from 80% of the total fish. This process was performed 20 times and the correlation between the predicted values for the final body weight and phenotypes was calculated in the validation group. A one-way ANOVA with repeated measurements was performed among the four groups (SNP–panel 1, SNP–panel 2, 30K MedFISH array and pedigree).

Results

In the validation group, the use of pedigree showed the lowest correlation coefficient (0.38) followed by the 30K MedFISH array (0.41), while the use of SNP–panel 1 (1,960 SNPs) provided the highest prediction (0.70) followed by the SNP–panel 2 (2,907 SNPs) in which the estimate was 0.66 (Figure 1). Moreover, a statistically significant difference (p-value<0.01) among the four genetic evaluation procedures was found. The post-hoc analyses with a Bonferroni adjustment were performed and revealed that there was a statistically significant difference between each pair.

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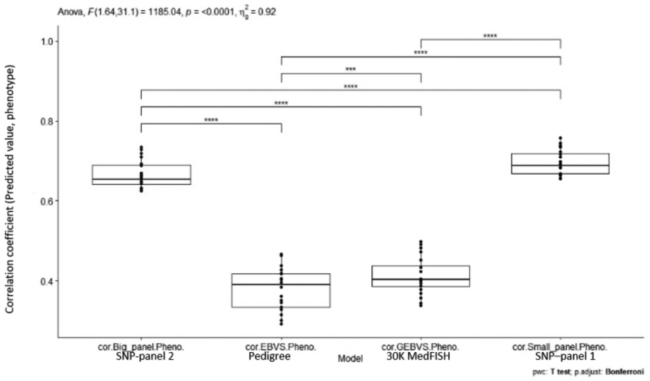

Figure 1. Boxplot for the correlation coefficient between predicted value and final body weight and results from the one-way ANOVA with repeated measurements among the four groups.

Discussion

When, selecting for disease resistance (parasite count) instead of body weight, the SNP – panel 2 provided the highest estimate (0.81) followed by the SNP – panel 1 (0.75) (Oikonomou et al. 2022b) while selecting for body weight the SNP-panel 1 provided the highest estimation (0.70). Regardless of the selection trait (disease resistance or body weight), higher correlation coefficient was established using small, selected SNP panels compared to the 30K MedFISH array.

Furthermore, higher correlation coefficient was found when using genomic information (30K MedFISH array and SNP-panel 1& 2) than when using pedigree relationship matrix. These findings indicate that small and cleverly selected SNP panels, such as SNP – panel 1 and 2 could potentially be utilized in cases of multi-trait genomic evaluation, and in some cases, can provide better predictions especially when parts of phenotypic information is missing.

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A PRELIMINARY LINKAGE AND QTL ANALYSIS FOR JAW DEFORMITIES IN GILTHEAD SEABREAM

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Introduction

Morpho-anatomical abnormalities is a critical issue in terms of unpredictability, product quality and animal welfare that requires continuous awareness in fish production (Koumoundouros 2010). Anomalies could be developed due to unfavorable abiotic conditions, inappropriate nutrition, and genetic factors (Boglione et al., 2013). Hence, their early detection is long ago considered of immense importance and selective breeding is widely recognized as a key factor for the development of resource efficient, sustainable, and economically profitable aquaculture productions (FAO 1995). Recently, Fragkoulis et al. (2018) studied the genetic parameters of the upper-jaw abnormalities in gilthead seabream and showed a low range of heritability (0.01-0.22), especially the heritability of the Inwards bending (bMa) and Fusion (FMaPm) of the jaw, were 0.04 and 0.22, respectively. In the present study a preliminary analysis was performed to identify microsatellite markers, from two linkage groups, affecting the Inwards bending (bMa) and Fusion (FMaPm) of the jaw.

Materials and methods

Genomic DNA from 498 offspring was extracted using a selective genotyping strategy based on the total length and /or their high phenotypic variation of the deformities. The selected offspring belonged to 153 full sib families, from 53 dams and 45 sires. All fish were genotyped using the microsatellite loci in LG1 and LG21 which were described by Loukovitis et al. (2016). Alleles were sized and individuals were genotyped using the software STRand 2.4.110 (http://www.vgl.ucdavis.edu/informatics/STRand). Offspring at the age of 39 Days Post Hatching, were examined for Inwards bending (bMa) and Fusion (FMaPm) deformations, as described by Fragkoulis et al. (2018). The phenotypes were analyzed as binary data (1: Absent of deformation, 2: Presents of Deformation). For the Inwards bending (bMa) and Fusion (FMaPm), 28.7% and 26.31% of the offspring showed deformation, respectively. A de novo linkage analysis was performed using the options “prepare”, “build” and finally the “flipsn” of CRI-MAP v3.0 software (Green et al., 1990). The linkage distances were estimated assuming the Kosambi’s mapping function. Furthermore, Quantitative Trait Loci (QTL) analysis was performed in QXPAK 5.0 software (Pérez-Enciso and Misztal, 2011), using a maximum likelihood Variance Component Analysis with the presence of a polygenic component (pedigree-based animal model) and the QTL effect.

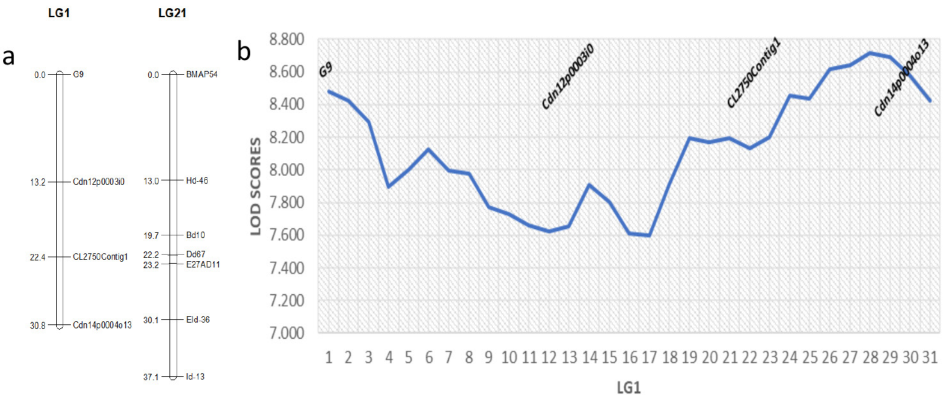

Fig 1 (a) Linkage groups 1 and 21 named according to Loukovitis et al. 2016 (b) Quantitative trait loci (QTL) analysis of bMa in LG1

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Results and Discussion

The markers were distributed into two linkage groups (LG1, LG21) named according to Loukovitis et al. (2016). The constructed map showed small differences between Loukovitis et al. (2016) and Tsiggenopoulos et al. (2014). In our study, the length of the LG1 and LG21 were 30.8cM and 37.1cM while in Loukovitis et al. (2016) were 23.5cM and 17.2cM respectively. Focusing on QTL analysis, no QTL related to the Fusion (FMaPm) was found on neither of the linkage groups studied, even though, Fragoulis et al. (2018) estimated an inheritance of the trait equal to 0.22 using an animal model. On the other hand, a QTL related to Inwards bending (bMa) was detected on LG1 linked to *Cdn14p0004o13* and its heritability was 0.11. For the Inwards bending (bMa) the related QTL must be investigated deeply, in order to examine its presence and clarify its effect, using a higher sample size before it can be used in Marker Assisted Selection.

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STRUCTURAL ENRICHMENT EFFECTS ON THE WELFARE OF GILTHEAD SEABREAM (*Sparus aurata*) BROODSTOCK

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In the last decade, the intensification of aquaculture practices, increasing number of facilities, number of species produced and rearing densities, have led to environmental stress, reduction of water quality, increase of chronic stress, growth impairment and health problems, all indicators of the reduction of the welfare of farmed fish (Saraiva *et al*., 2019). Recently, one of the tools that has been recommended to guarantee or improve the welfare of captive fish is the application of environmental enrichment (EE) (Brydges & Braithwaite, 2021). Structural, sensory, social, nutritional, or occupational EE aim to provide an adequate breeding environment, reduce stress to a minimum and meet the behavioural needs of individuals, promoting good welfare (Näslund & Johnsson, 2016).

For instance, structural enrichment improved survival rates and the physiological stress response, reduced stereotypical behaviour and aggressiveness, and enhanced cognition, exploratory behaviour, and brain physiological functions, among other positive effects on fish welfare (Neto & Giaquinto, 2020; Arechavala-Lopez *et al*., 2019, 2020; Räihä *et al*., 2019). However, most of these studies are focused on early-life fish stages or on-growing phases and there is still a lack of knowledge particularly on the broodstock. In this study, we measured behavioural and physiological indicators to assess the effects of structural environmental enrichment on the welfare of gilthead seabream broodstock.

Over the course of 5 months, 60 adult seabreams, distributed in six 3,000 L cylindrical tanks, were filmed regularly before, during and after feeding, cleaning, and sampling procedures. Three of the tanks were enriched with nine hanging organic ropes on 1 m^2 floating structures, while the other three tanks had no enrichment (control). Operational welfare indicators (OWIs) recently developed for farmed seabream were used and adapted to build an ethogram for the broodstock behaviour analysis.

Overall, fish in enriched tanks present a darker colour and perform more aggressive displays (erect fins, chases and flight behaviours, $p<0.001$). The enrichment structures also influence the swimming activity in the tank, since fish in enriched tanks behave more independently and vigorously ($p<0.001$ and $p=0.045$, respectively), and forage more actively ($p=0.014$), while fish in control tanks seem to school more ($p<0.001$). Moreover, fish on enriched tanks presented a significantly higher final weight ($p=0.049$) than fish from control tanks. The feeding event also had an effect on behaviour, especially on the spatial distribution of the fish in the tanks ($p=0.045$ for horizontal distribution; $p=0.013$ and $p=0.011$ for vertical distribution, respectively). Physiological analysis of cortisol are in process, which will allow us to check the effects of structural enrichment on stress hormones of seabream broodstocks.

It should be highlighted that this is the first study carried out on seabream broodstock, where we demonstrate that simple structural enrichment can induce positive changes on fish behaviour and welfare without major changes in rearing protocols, and therefore, they might be implemented at larger scale.

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EFFECTS OF OCEAN ACIDIFICATION AND WARMING ON CHEMICAL COMPOSITION, FITNESS AND ENERGY BUDGET OF JUVENILE SENEGALESE SOLE (*Solea senegalensis*)

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Introduction

Rising levels of atmospheric carbon dioxide are driving ocean warming and acidification, which may negatively affect the nutritional quality and physiological performance of marine species, with great economic importance to both fisheries and aquaculture sectors (Anacleto et al., 2018; Lah et al., 2018; Camacho et al., 2020). Yet, the impacts and interactions of both environmental stressors on nutritional properties, as well as on physiological (metabolic) responses, of most fish species remain unclear and still require further understanding. In this context, the aim of this work was to evaluate the effect of ocean acidification (i.e., ΔpH=−0.3 units equivalent to $\Delta p\mathrm{CO}_2$~+600 µatm) and warming (i.e., ΔT=+ 4 °C) (combined or not) on the chemical composition, fitness and energy budget of juvenile Senegalese sole (*Solea senegalensis*), a farmed species with high commercial value.

Materials and methods

Juvenile Senegalese sole (25.2±5.1 g total weight; 12.7±1.3 cm total length) were distributed and maintained in 12 tanks (4 treatments x 3 replicate tanks; n=4 individuals per tank) with independent recirculation aquaculture systems. The specimens were acclimated for one month and exposed to four scenarios during 75 days to understand the potential impacts under current and future expected conditions, according to projections of the Intergovernmental Panel on Climate Change (RCP 8.5 scenario; IPCC, 2021): i) Control (CTR) - seawater temperature set at 19 °C and pH at 8.0 ($p\mathrm{CO}_2$~400 µatm) (current conditions in Iberian Peninsula Senegalese sole farms); ii) Ocean acidification (OA) - seawater temperature set at 19 °C and pH at 7.7 ($p\mathrm{CO}_2$ ~1000 µatm); iii) Ocean warming (OW) - seawater temperature set at 23 °C and pH at 8.0 ($p\mathrm{CO}_2$ ~400 µatm); and iv) Ocean acidification and warming (OAW) - seawater temperature set at 23 °C and pH at 7.7 ($p\mathrm{CO}_2$ ~1000 µatm). Throughout the experimental period, fish were daily fed with 2% of their average body weight (divided by two meals). At the end of the trial, the ammonia-N, oxygen consumption rates (OCR) and biometric data were assessed. Then, fish were dissected to weight viscera and also to collect faeces (for energy determination). Moisture, ash, free fat, crude protein and gross energy were determined (at the beginning and end of the assays) in the whole fish body. Different calculations were performed to assess animal fitness, growth performance and energy budget.

Results and conclusions

Generally, the chemical composition, survival rate, fulton's condition factor and hepatosomatic and viscerosomatic indexes were not significantly affected by acidification and warming (acting alone or in combination), neither by sampling time. On the other hand, the total weight and total length (TL) significantly increased in all scenarios after 75 days. Weight gain of fish subjected to OA, OW and OAW was significantly higher than that obtained in fish exposed to CTR conditions. Furthermore, the highest relative growth rates (RGR) and specific growth rates in terms of wet weight (SGRw) and protein (SGRp) were observed in fish exposed to OW and OAW. In addition, the RGR and SGRp found in OAW were also significantly higher than those observed in OA. The TL was similar in the four treatments at the end of the experiment. Moreover, fish exposed to CTR conditions had significantly higher ammonia excretion rate (AER) compared to those exposed to OA, OW and

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OAW. However, all values found were very low (≤ 0.020 mg g^{-1} L^{-1} h^{-1}). Additionally, the maximum and standard metabolic rates (MMRs and SMRs), as well as energy intake (C), were generally significantly higher in fish subjected to OA, OW and OAW than in fish exposed to CTR conditions. Regarding energy distribution, the highest fraction was generally allocated to growth (48-63%), followed by excretion through faeces (36-51%), respiration (approximately 1%) and finally ammonia excretion (0.1-0.2%) in all treatments.

The results obtained clearly show that i) the juvenile Senegalese sole is resilient under seawater acidification and/or warming conditions; ii) changes in seawater pH and temperature values do not seem to have direct consequences on chemical composition, TL and animal fitness; iii) the acidification and warming (acting alone or in combination) have significant effects on growth (in terms of weight, RGR, SGRw and SGRp; more evident for OAW), AER, OCR and energy budget of juvenile Senegalese sole after 75 days of exposure. Thus, the impact of higher temperature and pH values (i.e., above the tolerance of this valuable commercial fish species produced in Europe) on its nutritional quality and physiological performance should be tested in coming studies.

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BLOOD BIOCHEMICAL PARAMETERS AS POTENTIAL NON-LETHAL BIOMARKERS FOR MEAGRE (*Argyrosomus regius*) IN AQUACULTURE

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Introduction

The development of new functional diets for Meagre (*Argyrosomus regius*) that promote both growth and health is an important step towards the increase of this species' production and has been the focus of various studies. However, analyses of fish welfare can be complex and either require animal sacrifice or end up causing additional stress for the fish. Therefore, it becomes important not only to find proper techniques and biomarkers that allow a better measurement of the effects of new diets on fish but also to find non-lethal biomarkers that will reduce the need for animal stress and sacrifice. The aim of this study was to obtain reference values and potential non-lethal biomarkers from blood samples of fish that will help assess fish nutritional status.

Methods

A nutritional challenge was performed for 7 weeks with groups juvenile meagre fish. Diets were tested in triplicate: an optimum diet (55.1 % of fish meal, FM) that was used as control (CTRL) and two other diets, a challenging diet (15% FM, CD) and an extremely challenging diet (5% FM, ED), partially devoid of fisheries-derived ingredients. At the end of the trial, 18 fish from each tank were collected. Blood samples were analysed using a Siemens epoc® Blood Analysis System to obtain biochemical parameters. Blood was also used for two different dilutions of HBSS and heparin (20U) solution (1:20 and 1:200) and immediately applied in a Neubauer chamber under x40 optic microscope ampliation for red blood cell (RBC) and total white blood cell (WBC) counts. The remaining blood was used to collect plasma following centrifugation (10,000×g, 5 min, room temperature). Plasma anti-protease and peroxidase activities, and total immunoglobulins and plasma protein were determined as described in Guardiola *et al*. (2018). Results were analysed using IBM SPSS statistics 27.0.1 software package (SPSS® IBM, Chicago, IL, USA). All data was analyzed by one-way ANOVA and Tukey's multiple range test was used for identifying differences between means. The probability level of 0.05 was used for the rejection of null hypothesis.

Results

Final weight, weight gain, feed intake, daily growth index, and feed efficiency decreased significantly with the increase of the diet's challenge level (Table 1). Blood parameters and plasma immunological and biochemical parameters showed significant differences between fish fed different diets, except for peroxidase and immunoglobulin levels, which showed no significant differences between fish fed different diets (Table 1).

Conclusion

Growth parameters were greatly affected by the reduction of diet quality, serving as good indicators of possible health issues in the fish and making samples collected from this trial ideal for the establishment of potential biomarkers. Results obtained from the analysis of the samples showed that blood parameters were affected by the challenging diets. This way, several of the parameters measured in fish blood at the end of the nutritional challenge showed potential as non-lethal biomarkers and seem to accurately indicate differences in fish fed lower quality diets. It is important to highlight parameters such as glucose, lactate and creatinine levels as parameters that we can easily connect to other physiological parameters of the fish and use collectively as biomarkers. These biomarkers can provide an initial view of the physiological status of the fish and can be later connected to other confirming biomarkers from fish samples.

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Table 1. Growth performance of fish fed the different diets (average initial body weight 4.6 g) and blood and plasma biochemical and immunological parameters of fish fed different diets.

		CTRL	CD	ED	p-value
Zootechnical performance	Final weight (g)	48.7 ± 1.0^a	30.0 ± 0.6^b	19.3 ± 1.0^c	<0.001
	Weight gain (g)	44.2 ± 1.0^a	25.4 ± 0.6^b	14.9 ± 1.1^c	<0.001
	Feed Intake (g MS/per fish)	52.0 ± 0.9	33.4 ± 1.1^b	28.0 ± 0.7^c	<0.001
	Daily growth index	3.99 ± 0.05^a	2.90 ± 0.04^b	2.07 ± 0.11^c	<0.001
Blood cell counts	WBC (%)	5.2 ± 2.0^a	7.6 ± 3.1^b	9.0 ± 4.2^b	<0.001
	RBC (%)	94.8 ± 2.0^b	92.4 ± 3.1^a	91.1 ± 4.2^a	<0.001
	TBC (10^6/uL)	1.2 ± 0.4^b	0.8 ± 0.3^a	0.7 ± 0.2^a	<0.001
Immune parameters	Peroxidase (U/mL)	43.8 ± 24.1	57.7 ± 27.4	59.8 ± 28.4	0.182
	Protease (% non-inhibited trypsin)	9.03 ± 0.89^b	7.73 ± 0.87^a	7.31 ± 0.74^a	<0.001
	Antiprotease (% inhibited trypsin)	52.8 ± 3.0^b	53.8 ± 4.1^b	47.7 ± 7.7^a	0.002
	PP (mg/mL)	0.47 ± 0.06^b	0.42 ± 0.06^{ab}	0.40 ± 0.06^a	0.008
	Immunoglobulins (mg/mL)	0.20 ± 0.06	0.20 ± 0.06	0.19 ± 0.07	0.746
Biochemical parameters	pH	7.11 ± 0.06^a	7.17 ± 0.04^b	7.09 ± 0.05^a	<0.001
	pCO_2 (mmHg)	14.0 ± 1.2^b	12.9 ± 1.2^a	12.7 ± 0.1^a	0.001
	pO_2 (mmHg)	37.9 ± 23.3^a	32.4 ± 10.2^a	63.8 ± 48.1^b	0.008
	K^+ (mmol/L)	4.1 ± 0.3^a	4.5 ± 0.4^b	4.3 ± 0.5^{ab}	0.008
	Ca^{++} (mmol/L)	1.4 ± 0.1^b	1.3 ± 0.1^a	1.2 ± 0.2^a	<0.001
	Glucose (mg/dL)	163.5 ± 41.3^c	77.4 ± 17.7^b	49.4 ± 11.6^a	<0.001
	Lactate (mmol/L)	4.8 ± 1.6^b	3.80 ± 1.2^{ab}	3.0 ± 0.8^a	0.001
	Creatinine (mg/dL)	1.1 ± 0.6^b	0.72 ± 0.3^{ab}	0.6 ± 0.1^a	<0.001

Mean values and standard deviation (±SD) are presented for each parameter (n=3, 6 pools of 3 fish/diet).
Different letters in the same row stand for statistical differences between diets ($P < 0.05$).
Daily growth index: ((final body weight$^{1/3}$ – initial body weight$^{1/3}$) / time in days) x 100
Feed efficiency: wet weight gain/ dry feed intake
PP: Plasma protein; WBC: White blood cells; RBC: Red blood cells; TBC: Total blood cells; Diets: CTRL: Control; CD: Challenge; ED: Extreme challenge

PRELIMINARY DATA ON HISTOLOGICAL BIOMARKERS OF MALNUTRITION IN FISH

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Introduction

In recent years the development of new diets for aquaculture fish species aimed to avoid the inclusion of high amounts of fisheries by-products, focusing on the use of alternative ingredients that will provide growth, health, environmental and economic benefits. The effects of such diets on Meagre (*Argyrosomus regius*) have been the focus of various studies though it is often difficult to observe long-term effects on fish health and detect them in zootechnical performance. Consequently, it is essential to establish biomarkers that are precise and more practical to measure, allowing easier observation of the effects of diets on fish. The aim of this study was to obtain reliable biomarkers from histological analysis of fish intestine, with focus on objective measures that do not require an extensive background in intestinal histology, that will assess fish health in response to diets. For this, a semi-quantitative analysis was combined with a quantitative analysis and machine learning methods to mine for more precise analytical methods that will facilitate the histological analysis for future research.

Methods

Nutritional challenge

A nutritional challenge was performed for 7 weeks with juvenile meagre. Diets were tested in triplicate: an optimum diet with fish meal (55.1 % of fish meal, FM) used as control (CTRL) and two other diets, a challenging diet (15% FM, CD) and an extremely challenging diet (5% FM, ED) partially devoid of fisheries-derived ingredients. At the end of the trial, 18 fish from each tank were sampled and the intestine was removed and divided into anterior, intermediate and distal intestine for further histological analysis.

Histological analysis

The three intestinal sections were processed and sectioned using standard histological techniques. Sections were stained with hematoxylin and eosin. Both quantitative and semi-quantitative analysis were performed with ZEISS software ZEN 3.5 (blue edition).

Statistical analysis

Results were analyzed using IBM SPSS statistics 27.0.1 software package (SPSS® IBM, Chicago, IL, USA). Quantitative data was analyzed by one-way ANOVA tests and Tukey's multiple range test was used for identifying differences between means. Semi-quantitative data was analysed by a nonparametric Kruskal–Wallis test followed by multiple comparisons. Probability level of 0.05 was used for rejection of null hypothesis.

Nutritional status classification using Decision Trees and K Nearest Neighbours classifiers

Supervised machine learning classification algorithms (Decision Trees, DT and K-Nearest Neighbours, KNN) were trained to predict the nutritional status of fish based on a vector of 18 intestinal histological features of semi-quantitative or quantitative nature. Briefly, data was split 70:30 into training:test datasets, respectively. DT was plotted with 10 depth levels, 10 features and using entropy as criterion for the best split (decision tree parameters were decided after cross-validation). KNN was performed after data normalization, with K=4 neighbours. All analysis were run with Python 3 using pandas, numpy, seaborn, matplotlib and slkearn libraries.

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Results

Semi-quantitative

In the anterior intestine the parameters presence and vacuole size, number of goblet cells, lymphocytes in the epithelium (p=0.000) and granulocyte localization (p=0.046) showed significant differences between fish fed different diets, the parameters of villi fusion (p=0.940), lamina propria size (p=0.647) and submucosa inflammation (p=0.131) did not present significant differences between groups. For the intermediate intestine the parameters that showed significant differences were: villi fusion(p=0.15), granulocyte localization (p=0.006), lamina propria size, submucosa inflammation, presence and vacuole size, number of goblet cells and lymphocytes in the epithelium (p=0.000). Finally, in the distal intestine section, all previously mentioned parameters showed significant differences between fish fed different diets (p=0.000).

Quantitative

In the anterior intestine quantitative parameters that showed significant differences between fish fed different diets were: villi number (p=0.001), lumen area (p=0.039), total area, maximum total diameter, villi area and villi density (p=0.000). Maximum lumen diameter did not present significant differences between groups (p=0.059). In the intermediate intestine all previously mentioned parameters showed significant differences between groups (p=0.000) and the same was observed in the distal intestine section with p=0.000 in all parameters except villi number (p=0.028), total area and total maximum diameter (p=0.001).

Nutritional status classification

DT algorithm was able to predict with 64% accuracy the nutritional status of the fish. Fish subjected to the extreme treatments (optimum diet and extreme diet) were more precisely identified (66% and 69% precision, respectively). KNN returned an accuracy of 57% with the extreme diet being the one classified with higher precision (65%). Both methods used a combination of quantitative and semi-quantitative histological features, but DT performed better compared to KNN.

Conclusion:

Combining semi-quantitative histological features with quantitative measures of intestinal structure can be regarded as an important biomarker to predict fish nutritional status. Preliminary data analysis showed an interesting level of accuracy, but far from optimal. Based on our preliminary data, histology biomarkers should be combined with other analysis to fully discriminate fish nutritional status. Further data analysis between intestinal sections and within type of analysis (quantitative vs semi-quantitative) will be performed with the goal of establishing histological biomarkers of nutritional status that are simple, reliable and do not require extensive experience in histological analysis to identify sub-optimal nutritional status.

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COMBINING ALKALINE WITH AUTOCLAVING TREATMENT FOR THE IMPLEMENTATION OF *Codium tomentosum* IN DIETS FOR EUROPEAN SEABASS (*Dicentrarchus labrax)*

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Introduction

Morden aquaculture has been decoupled from using fish meal as one of the major ingredients for aquafeeds. Many aquafeed producers now use soy and corn-based proteins, often associated with sub-optimal growth and feed efficiency, increased land and water use, and aquaculture carbon-foot print. These non-local resources have generated new ecological and socio-economic issues [1].

Macroalgae are a readily available resource with moderate protein and high fiber, minerals, antioxidants, and vitamin content. Production of macroalgae does not rely on land and freshwater use and may also be harvested from coastal regions with minimal environmental impact. Several authors have studied the inclusion of macroalgae in diets, concluding that high (>10%) dietary incorporation of macroalgae negatively affected growth and feed utilization parameters. Authors support that disruption of the macroalgae cell wall may increase nutrient bioaccessibility and access to the digestive enzymes, favoring nutrient absorption efficiency and fish growth. Adequate physical, chemical, enzymatic, and/or fermentation treatments may be required to disrupt the macroalgae extracellular polysaccharides matrix [2,3].

Codium tomentosum natural distribution included Europe and North Africa. It has also been successfully produced as an extractive species in integrated multi-trophic aquaculture systems (IMTA), including in Portugal. *Codium tomentosum* has a relatively higher protein level, circa 20-25%, but its potential as an aquafeed ingredient has been little studied. The present work aims to investigate the potential of alkaline hydrothermal pre-treatment of *Codium tomentosum* to increase its viability as an ingredient in diets for European seabass.

Material and Methods

Treatment of macroalgae

Samples of *Codium tomentosum* were provided from AlgaPlus, Aveiro, Portugal. *Codium tomentosum* was mixed with water (control) or NaOH (0.5N and 1N; solid-liquid ratio of 4:3) and autoclaved (121ºC) for 30 and 60 min. All the samples were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein, soluble protein, and phenols.

Growth trial

Four experimental diets were formulated to be isoproteic (48% dry matter basis) and isolipid (18% dry matter basis). A practical diet was used as a control diet (D1), and three other diets were formulated to include 7.5% *Codium tomentosum* at the expense of a mixture of soybean and wheat meal, namely non-treated *Codium* (D2), autoclaved for 30 min with 1N NaOH (D3), and autoclaved for 60 min with 1N NaOH (D4). The growth trial was conducted in a RAS system equipped with 12 tanks of 500 L water capacity, thermoregulated to 22 ºC. 192 acclimatized European seabass juveniles (IBW of 38 g) were divided into 12 groups and fed each experimental diet in triplicate, twice a day until satiation, 6 days a week, for 11 weeks.

Results

Irrespective of autoclave time exposure, *Codium tomentosum* soluble protein, and phenols content were significantly increased, while crude protein, NDF, and ADF significantly decreased when mixed with NaOH 1N than with NaOH 0.5N. These results suggested that the combined alkaline (NaOH 1N) with hydrothermal treatment (for 30 or 60 min) disrupted the cell wall of macroalgae being chosen to be incorporated as an ingredient in diets for European seabass.

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At the growth trial, it was observed that all the diets were well accepted by the fish, and mortality was low and not influenced by the diets. Dietary inclusion of 7.5% of untreated *Codium tomentosum* did not affect overall growth performance, feed intake, and feed utilization efficiency. However, relative to the control diet, the dietary inclusion of 7.5% *Codium tomentosum* treated with NaOH 1N and autoclaved for 30 min significantly increased growth performance and feed and protein efficiency ratio. In contrast, the *Codium tomentosum* treated with NaOH 1N and autoclaved for 60 min resulted in similar growth performance and feed utilization efficiency to the control diet, which was significantly lower than that obtained for the *Codium tomentosum* treated with NaOH 1N and autoclaved for 30.

In conclusion, the pre-treatment of *Codium tomentosum* with NaOH 1N and autoclaved for 30min significantly improved the potential of *Codium tomentosum* as a local and complementary feed ingredient for carnivorous fish species.

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EFFECTS OF DIFFERENT FULL-FAT *Hermetia illucens* PREPUPAE MEAL DIETARY INCLUSIONS ON REPRODUCTIVE PERFORMANCES OF ADULT FEMALE ZEBRAFISH

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Introduction

The quality of nutrients in broodstock diet represents one of the major factors influencing fish reproductive success and offspring quality/survival. Especially for mature female fish, reproduction is one of the most metabolic demanding activities and relies on the proper administration of dietary protein and lipids, especially fatty acids. Specifically, polyunsaturated fatty acids (PUFA) are drained from storage sites (mainly muscle and liver) to the ovary where can be involved in follicle maturation or incorporated into the eggs as the main nutritional source for the embryo while guaranteeing proper hatching and early larval development. For that reason, over the last years, great attention has been given to the role of specific dietary nutrients, such as PUFA, in the reproductive processes of teleosts. However, in cultured fish, several stressors, including malnutrition, can potentially interfere with oogenesis and induce atresia of ovarian follicles. For this reason, investigating whether a diet is able to correctly sustain fish reproduction is of primary importance, especially when alternatives to conventional ingredients are tested. On this regard, insects gained great attention within aquafeeds formulation thanks to their eco-friendly and cost-effective rearing and their promising nutritional values. In particular, the Black Soldier Fly (*Hermetia illucens*; BSF) is characterized by a balanced essential amino acid profile comparable to fish meal and represents a valuable protein source for fish diets. However, the BSF fatty acids profile, rich in saturated fatty acids (SFA) and poor in PUFA is not always ideal for fish, especially when considering fish lipid requirements during the critical life-cycle stages of oocyte maturation and reproduction. The present study was aimed to test the effects of two different dietary inclusion levels (25 and 50 %) of full-fat BSF prepupae meal respect to fish meal on reproductive performances of adult zebrafish females through a multidisciplinary approach.

Zebrafish (*Danio rerio*) is a well-established experimental model for studies on reproduction since it is easily reared in laboratory conditions and, if compared to other teleosts species, it reaches sexual maturity in a relatively short time. On this regard, zebrafish represents an ideal organism to better understand the link between broodstock nutrition and female reproductive success, generalizing how several biological processes take place in model organisms and thus providing useful information for finfish production. The well-consolidated knowledge about zebrafish oocyte development and maturation is essential to better understand the dietary effects of new diets.

Materials and Methods

A control diet containing fish meal, wheat gluten meal, wheat flour, pea protein concentrates and FO as major ingredients, was prepared according to a commercially available standard diet for zebrafish (Zebrafeed, Sparos ltd, Portugal). BSF-based diets were prepared by including 25 and 50 % of full-fat BSF prepupae meal respect to FM (referred as BSF25 and BSF50, respectively) in the control diet formulation. All the experimental diets were iso-nitrogenous (40%), iso-lipidic (17%) and iso-energetics (22%). Zebrafish were fed the experimental diets for 12 months (form larvae to adult) and the reproductive performances of mature females were assessed through a multidisciplinary approach including: (i) evaluation of growth, gonadosomatic index, spawning and hatching rates: (ii) gas-chromatographic analyses for fatty acids composition of adult females carcasses and fertilized egg; (iii) histological analysis on the ovary to assess oocyte maturation stages and the oil droplet percentage in class IV oocytes; (iv) spectroscopic analysis by Fourier Transform Infrared Microspectroscopy (FTIRM) to analyse the macromolecular composition of class IV oocytes; (v) molecular analyses on liver samples to evaluate the expression of genes involved in PUFA biosynthesis (*elovl2*, *elovl5*, and *fads2*).

Results and Discussion

The inclusion of BSF prepupae resulted in a dietary PUFA reduction and a parallel increase in SFA. This unbalanced dietary lipid profile also characterized the fatty acid composition of females' carcasses but did not affect their growth and gonadosomatic index. Differently, considering class IV oocytes and fertilized eggs, a higher SFA and lower PUFA content were only detected in BSF50 group by both gas chromatographic and spectroscopic analyses. Accordingly, in this study, fish fed BSF diets (particularly BSF50) showed a higher expression of genes involved in PUFA biosynthesis able to convert

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shorter-chain precursors (moved from storage sites like muscle and liver) to highly unsaturated ones. The different trend in PUFA content between the fish carcasses and the spawned eggs or class IV oocytes represents a very interesting result, which underlines a significant investment of female zebrafish in the reproductive event, strongly suggesting the selective accumulation of these fatty acids in the eggs. Adult female zebrafish were perfectly able to cope with the lower (25%) dietary BSF prepupae meal inclusion level respect to fish meal, resulting in similar reproductive performances compared to Control. Conversely, the BSF50 group showed a general decrease in spawned eggs, possibly due to a delay in oocyte maturation (greater abundance of previtellogenic oocytes detected by histological analyses) caused by (i) the necessity of a longer ex novo PUFA biosynthesis by the fish and subsequent oocyte accumulation or (ii) the activation of atretic processes. This last hypothesis is supported by the increase in atretic oocytes and by a reduction of postovulatory follicles in BSF50 with respect to BSF25 and Control. However, the BSF50 group showed a comparable hatching rate to that observed in Control and BSF25 ones. This is one more evidence of the extraordinary effort that female fish put in the reproductive event, choosing for quality rather than quantity by activating specific processes to select those oocytes that should be ovulated or reabsorbed.

In conclusion, the results obtained in this study showed that the dietary substitution of FM with a full-fat BSF prepupae meal up to 25% did not affect reproductive performances of female zebrafish, while a higher substitution (50% with respect to FM) resulted in reproductive impairments, specifically in terms of number of spawned eggs.

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ALTANET – THE ATLANTIC LOW TROPHIC AQUACULTURE NETWORK

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Introduction

Low trophic aquaculture is growing in importance, and the number of industry actors, species, products, projects, events, and publications is increasing. The goal of the Atlantic Low Trophic Aquaculture Network (ALTANet) is to create and maintain an overview of LTA-related news stories, events, projects, calls, tenders, reports, publications, training materials, and websites. The main output from the network is the altanet.info dynamic website which contains links to content relating to aquaculture involving macroalgae, echinoderms, molluscs, and crustaceans, as well as Integrated Multi-Trophic Aquaculture (IMTA) in general.

Manual updates and machine learning algorithms for dynamically generated LTA-related content

The EU-projects AquaVitae and ASTRAL, with supporting funding from the Norwegian Research Council, initiated the ALTANet network and in particular created the altanet.info website. The website has two component parts; one based on manual updates and one based on dynamically generated content created by a machine learning algorithm. The manual updates are done by correspondents, and relate to existing and upcoming events, projects, reports, publications, videos, and webinars. Anyone interested in adding information about a LTA-related resource can do so (or ask a correspondent to do so) and based on this input searchable and sortable ordered lists are created where the content is accessible through filters and free text search. The dynamic content is generated by a machine learning algorithm that runs every night to trawl the internet for LTA-related news stories. An initial set of LTA-related keywords and rules were defined which yielded around 10 candidate news stories per day, of which around 5 were actually relevant. After manual classification of more than 1000 news stories (into relevant / not relevant) the accuracy of the algorithm increased (it compares candidate news stories with those known to be relevant) and it is now around 93%. The algorithm now runs without the manual classification step, which means that around 5 LTA-related news stories are added to the altanet.info website every day.

Discussion and conclusion

The altanet.info website can be useful for anyone interested in low trophic aquaculture in the following ways:

- As a communication channel used to highlight existing and upcoming events, projects, reports, publications, videos, and webinars, and to make sure that the LTA community knows about them
- As an openly available resource for searching for and finding out about existing and upcoming events, projects, reports, publications, videos, and webinars
- As a daily and dynamic provider of online LTA-related news from all over the world

All content on the altanet.info website is classified by species or production type, and the pre-defined species / production types are macroalgae / seaweed, echinoderms / sea urchins / sea cucumbers, molluscs / oysters / mussels, crustaceans, and Integrated Multi-Trophic Aquaculture (IMTA). All website content can be searched, selected, and filtered by species / production type, which means that anyone only interested only in one particular species can filter out all other content if desired.

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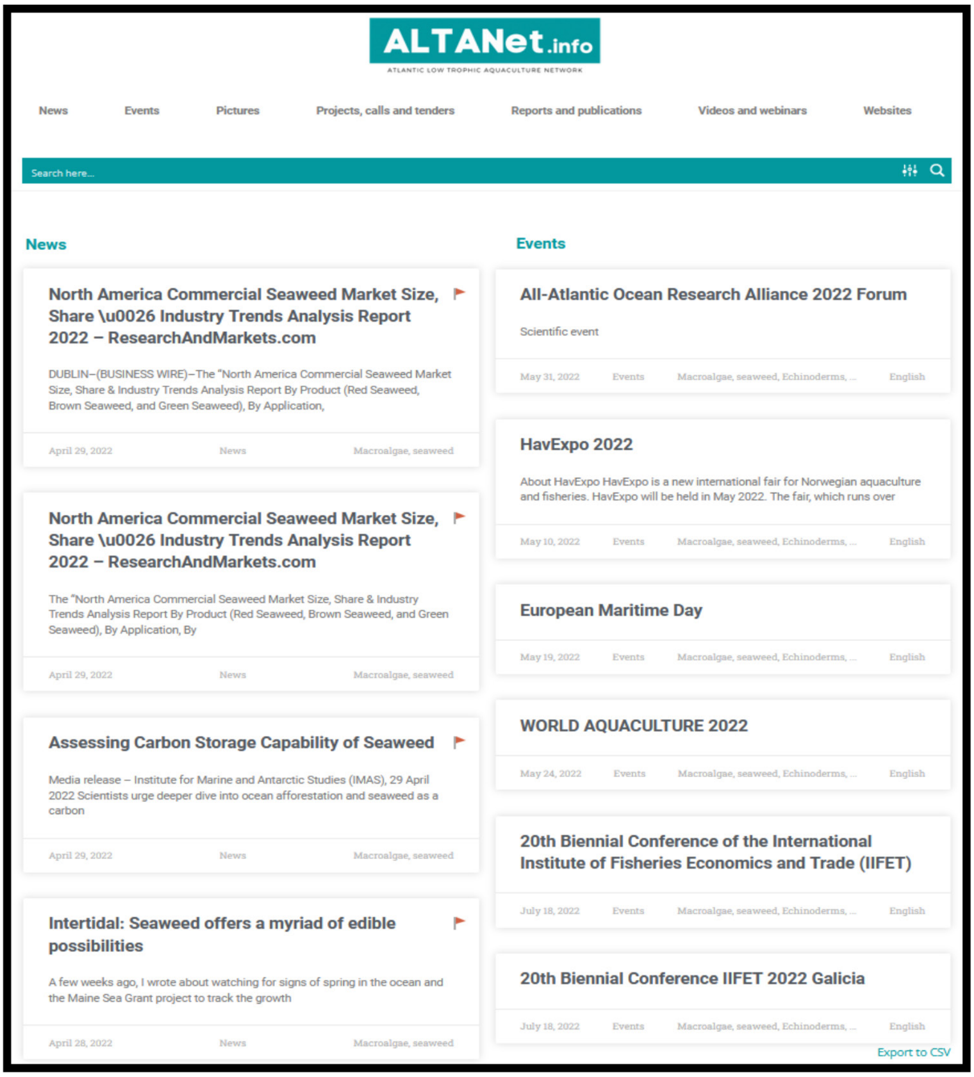

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Assessing Carbon Storage Capability of Seaweed

Media release – Institute for Marine and Antarctic Studies (IMAS), 29 April 2022 Scientists urge deeper dive into ocean afforestation and seaweed as a carbon

April 29, 2022 News Macroalgae, seaweed

Intertidal: Seaweed offers a myriad of edible possibilities

A few weeks ago, I wrote about watching for signs of spring in the ocean and the Maine Sea Grant project to track the growth

April 28, 2022 News Macroalgae, seaweed

Events

All-Atlantic Ocean Research Alliance 2022 Forum

Scientific event

May 31, 2022 Events Macroalgae, seaweed, Echinoderms, ... English

HavExpo 2022

About HavExpo HavExpo is a new international fair for Norwegian aquaculture and fisheries. HavExpo will be held in May 2022. The fair, which runs over

May 10, 2022 Events Macroalgae, seaweed, Echinoderms, ... English

European Maritime Day

May 19, 2022 Events Macroalgae, seaweed, Echinoderms, ... English

WORLD AQUACULTURE 2022

May 24, 2022 Events Macroalgae, seaweed, Echinoderms, ... English

20th Biennial Conference of the International Institute of Fisheries Economics and Trade (IIFET)

July 18, 2022 Events Macroalgae, seaweed, Echinoderms, ... English

20th Biennial Conference IIFET 2022 Galicia

July 18, 2022 Events Macroalgae, seaweed, Echinoderms, ... English

INCREASED PRODUCTION AND SOCIETAL EXPECTATIONS: THE IMPORTANCE OF SOCIAL ANCHORING IN NORWEGIAN SALMON AQUACULTURE INDUSTRY

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Introduction

The salmon aquaculture industry has attracted considerable controversy, and in later years, social and ecological challenges have caused limitations to industry growth in several salmon producing countries, including Norway (Young et al., 2019). Both industry and government share a vision of 5-fold increase in production within the next 30 years, however, this vision is also met with skepticism, especially concerning a potential increase in environmental challenges.

Recent regulatory measures and governmental instruments are largely focusing on solving environmental challenges as a way to improve the industry's sustainability. Environmental sustainability is (from the governmental perspective) described as something that is bearable by both nature and society, or in other words, acceptable for society. We therefore argue that this is closely related to public perception of the industry and its production, and thus, also including social and economic dimensions of sustainability. Public acceptance is an important element for the Norwegian salmon aquaculture industry and can be viewed as a form of social anchoring, on local and national level. Having public support for the industry is crucial in order to gain access to good production sites and for an increase in production, and in satisfying consumers in local and global markets.

From its beginning, the Norwegian salmon aquaculture industry has been regulated by various policy instruments and in line with important political objects, which has especially focused on increasing business activity in rural coastal communities. The last decade, however, we have seen a change in aquaculture policy moving away from specific political objectives and towards an industry policy relying to a greater extent on market forces. At the same time there has been an exceptional high profitability in the industry. So when public policies have been changing from a local to a more national priority in distributing industry benefits, local communities are less content with their share of value creation. Previous research on public attitudes, public acceptance, and social license is ambiguous on the various factors important for shaping public opinion, and thus, how to improve attitudes towards the salmon aquaculture industry on both a national and local level (Krøvel et al., 2019).

This paper investigates how public regulation and it's pertaining tools, incorporate measures that secures a social anchoring of the aquaculture industry. It takes as a departure point, the different schemes for production licenses, and how these schemes were interpreted by the public, as in specific stakeholders. We are examining to what extent these schemes appear to include elements that may (potentially) improve the social dimension of sustainability, in other words, specific measures to improve a social anchoring of industry activity on local and national level. The research questions of this paper is: What elements does the public consider important for the social sustainability of the aquaculture industry? And to what extent, does proposed schemes for increased production incorporate measures that respond to such concerns? And finally, are these topics brought forward in the consultation responses from different stakeholders?

Materials and methods

The empirical data for this paper is a combination of quantitative data; results from a nationwide survey (N = 1183), and qualitative data; document analysis. The survey was conducted in 2020 and asked people about their opinion on salmon aquaculture production and the industry presence in their local community. The qualitative analysis is based on publicly available material related to Norwegian aquaculture regulation in general, and documents concerning the development licenses, eco-technology licenses, and licenses for offshore aquaculture (not finalized until May 2nd) in particular, e.g. white papers, green paper, responses to consultation rounds for the three different licenses schemes.

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Results and discussion

This paper combines findings from social acceptance literature and a recent study of public attitudes towards the Norwegian salmon aquaculture with the current policy development which focuses on technology development for improving the industry's environmental sustainability. The survey results show that the general impression of the Norwegian salmon aquaculture industry is fairly good, however; people are reluctant to increase industry production, and it is reported that the industry is to a larger extent behaving in accordance with the expectations from the authorities rather than the expectations from the industry. Benefits like job creation, increases business activity, creating robust communities, and food production is emphasized as important contributions, but these does not outweigh the negative consequences sufficient for wanting production growth. In addition, previous studies and ongoing debates have also shown how expectations from coastal communities are concerned with the distribution of economic benefits. Findings from the survey suggest that people are not satisfied with the distribution of economic benefits, especially on a municipal level.

With the establishment of an Aquaculture Fund in 2016, higher governmental income when enabling growth (through the traffic light system), and a new production fee introduced in 2021, the coastal communities have increased their economic benefits from the industry. This is an important step for improving the social anchoring of industry on a local level. However, with the newly proposed license schemes, the focus on social and economic benefits for coastal communities seem to be displaced by the focus on development of technological innovations to solve environmental challenges.

The data collection for the new licensing schemes (analysis of hearing responses) is on-going through May-June 2022; preliminary results suggests that new governmental policies to a large extent focuses on improving environmental sustainability, and this have potential direct and indirect impacts also for the social and economic dimensions of sustainability. For example, the proposal for offshore aquaculture licenses mainly view economic and social revenues as beneficial for the national level. We find that an implication of these licenses is that with production moving out of the municipal sea area, this also distances the production and benefits from the local community. Revenues to coastal communities hosting aquaculture production might decrease, due to value added and employment being moved to more central regions. On the other hand, the development of the new technological solutions could lead to an increase in value and job creation in the supplier industry (ripple effects), although these may be localized elsewhere and without proximity to aquaculture companies and the coastal communities voicing their expectations about the industry presence.

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INTEGRATED MULTITROPHIC AQUACULTURE OF ABALONE *Haliotis midae* AND SEA CUCUMBER *Neostichopus grammatus*: AN EFFICIENT AND SUSTAINABLE METHOD TO REDUCE ASSOCIATED TANK CLEANING COST AND LABOUR INVOLVED IN ABALONE FARMING

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Abalone farming produces nutrient-rich sludge, and the frequent cleaning and removal of sludge from abalone tanks is labour and capital-intensive. This study aimed to assess the feasibility of abalone (*Haliotis midae*) and the warty sea cucumber (*Neostichopus grammatus*) in an IMTA system and the effect of reducing abalone tank cleaning frequency on abalone growth, water quality, and sludge content and volume.

The study was conducted for 16 weeks. Four treatments namely, abalone cocultured with sea cucumber with tanks cleaned once weekly (AS1); abalone cocultured with sea cucumber with tanks cleaned twice weekly (AS2); abalone only with tanks cleaned once weekly (A1); abalone only with tank cleaned twice weekly (A2) were used. Abalone were maintained under commercial stocking conditions and were fed Abfeed™ and seaweed (*Ulva lacinulata* and *Gracillaria gracilis*) while the sea cucumbers fed on the abalone faeces and left-over feed, which settled at the abalone tank's bottom (sludge). The coculture of abalone and sea cucumber is feasible with a 99±0.43% survival of abalone. There was a significant interaction between the type of culture and the frequency of cleaning ($F_{(1,476)} = 12.41$, $p<0.001$). Abalone in the A2 treatment group showed better growth ($p = 0.006$) than those of the A1 treatment group, while abalone in AS1 had significantly better growth ($p<0.001$) than those in the AS2 treatment group. Also, abalone in AS1 showed better growth ($p = 0.026$) than abalone in the A1 treatment. However, abalone in AS1 had similar growth ($p = 0.53$) with those in A2. This study shows that optimization of growth in abalone farming can be achieved by cleaning tanks twice instead of once weekly. However, when abalone are cocultured with sea cucumber, tanks need only be cleaned once a week without compromising abalone growth. The coculture of abalone and sea cucumber results in an additional aquacultured product, a reduction in the labour and associated cost of cleaning without an additional cost of production.

AUTONOMOUS, CONTINUOUS AND REACTIVE-FREE NUTRIENT AND MICRO-ALGAL MONITORING IN SEAWATER

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Introduction

The aquaculture is a very promising sector for the future of food production, but it also brings a lot of challenges to be sustainable and profitable. The early detection of harmful algae blooms (HAB) is one of them. Meeting it leads to the need for an autonomous and continuous monitoring of nutrients and micro-algal composition of the seawater.

Solutions for such a challenge has already been explored by, for example, using reagents and colorimetry analysis in an autonomous microfluidic lab. In this paper, we explain the concept and show some preliminary results of an affordable, reagent-free, multi-parameters and autonomous sensor specifically oriented toward the nutrient and micro-algae assessment. It is based on a MEMS spectrometer and an original illumination device coupled to AI models.

Concept of an autonomous muti-parameters water analyser

It is well established that a broad spectrum of physicochemical parameters (e.g., ammonium, nitrate, and dissolved oxygen) can be measured based on their reaction to UV-visible light absorption in the range of 200-800 nm. Moreover, the micro-algae present in the seawater are known to be fluorescent under specific illumination conditions. Consequently, for an early detection of HAB, it is interesting to perform continuous measurements of absorbance and fluorescence of the seawater near the fish containers to get very frequent measurements of nutrients and micro-algal composition. These frequent in-situ measurements can be sent to a cloud storage over a long-range network and provide a live monitoring of the water of the fish tank.

This brings high-level requirements for such a device like autonomy, low power consumption, affordability, and compact design. To meet them, the idea developed in the water analyser presented here is that all the light measurements can be done using a single illumination device designed to be able to do absorbance and fluorescence coupled to a small spectrometer.

MEMS spectrometer

Microelectromechanical systems (MEMS) processes allow to produce tiny mechanical and optical devices using techniques like the ones used in the production of micro-electronic chips. Recently, some manufacturers released spectrometers on the market made by this technology. They are very small (about 20x10x10 mm) lightweight (5 g) and consume less than 0.1 Watts for a decent spectral resolution (less than 10nm) but with a wavelength range limited to the visible spectrum (340 to 850nm). This makes them good candidates to be the basis of an autonomous water analyser.

First prototype

To evaluate the feasibility of an autonomous multi-parameter sensor based on this spectrometer, we coupled it with an original water illumination device to automatically perform absorbance and fluorescence measurements in a single instrument. This device holds the spectrometer on one side of a sample holder and presents holes on the other sides where various LED and lamps are inserted. The hole opposed to the spectrometer is used for absorbance illumination and the two others for fluorescence. On this prototype, one xenon lamp and 6 LED are distributed in these holes.

This first prototype was driven by a microcontroller and an interfacing electronic board that allow to pilot independently each lamp, and which also drive the spectrometer to get the data, pre-process them for background subtraction and store the results on a SD card. This process is run each time a button is pressed. The acquired spectra are then extracted using a companion software running on a computer to be processed further.

Calibration

The way to infer parameters measurements from the acquired spectra can be challenging, especially because of the relatively small spectrum band of the MEMS spectrometer but also because of the complex response of the seawater to illumination. However, it has been demonstrated that a deep learning approach can be very effective to extract information from a spectrum even when a chemometrics approach fails or gives poor results[1]. To do so, it is needed to gather a lot of data samples to train a model and evaluate its performances.

The prototype is running, and a data acquisition campaign is started and will continue during the coming months. The preliminary results will be shown at the conference.

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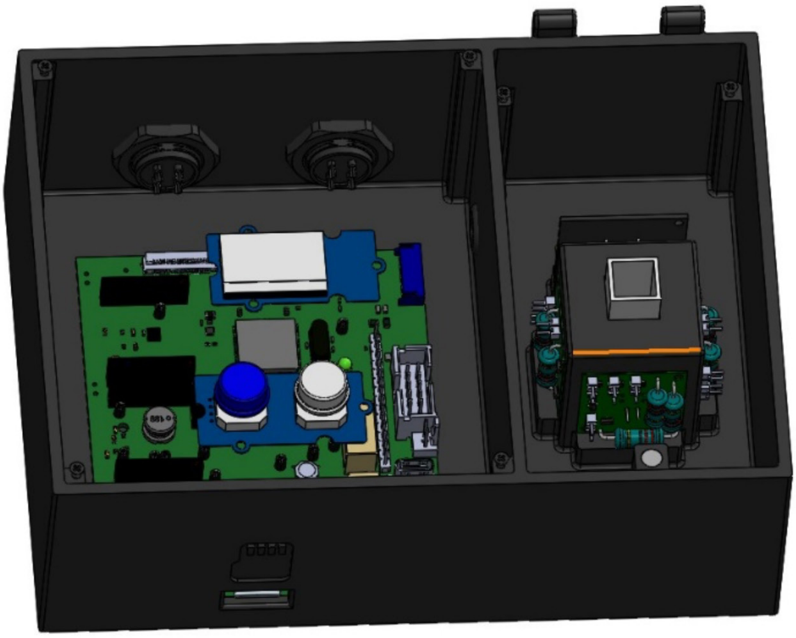

Figure: Exploded view of the first spectro-fluorometer prototype. Left: the electronic board with the buttons and screen. Right: the illumination device around the sample holder.

Acknowledgements

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OCCURRENCE OF ANTIBIOTIC RESISTANCE AND VIRULENCE OF GENUS V*IBRIO* IN BIVALVE AQUACULTURE OF EASTERN ADRIATIC

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Introduction

Bivalve filter water as part of feeding process and such accumulate large number of various particles and microorganisms. Most common bacteria genera found in marine environment is genus *Vibrio*. In this genus belong bivalve commensal, pathogens and opportunistic pathogens. Besides bivalve pathogens, in this genus we can find human pathogens popularly known as "big four" - *V. alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*, and they cause diseases – vibriosis (Baker-Austin et al, 2018). Thus, it is necessary to determine the structure of natural *Vibrio* community, including the occurrence of potential pathogens, in the eastern Adriatic bivalve aquaculture. Additionally, one of the growing problems in marine environments is the emergence of antibiotic resistance due to the excessive use of antibiotics and anthropogenic discharges into the marine environment. However, antibiotic resistance in eastern Adriatic bivalve aquaculture has been poorly studied (Baralla et al, 2021).

Materials and methods

Focus of our research are two most important species in aquaculture in Croatia - European flat oyster, *Ostrea edulis* Linnaeus, 1758 and Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819. These species were sampled in two protected marine reserves – Lim Bay and Mali Ston Bay, locations which are known for bivalve aquaculture. We determined physico-chemical parameters of seawater and also took samples of seawater, sediment and bivalve tissue (hepatopancreas and gills). Furthermore, culturable heterotrophic and total *Vibrio* bacteria in seawater, sediment and tissues were isolated on Marine agar and *Vibrio* selective TCBS medium on 22°C and 35°C. Additionally, we identified *Vibrio* clades with MALDI-TOF mass spectrometry (Culot et al, 2021) and species with MLSA (multilocus sequence alignment) method (Pascual et al, 2010; Pérez-Cataluña et al, 2016). All *Vibrio* isolates were tested for antibiotic resistance *via* disk diffusion on Mueller Hinton agar for 13 selected antibiotics which are frequently used in aquaculture and medicine. Our next step is to determinate differences between species, locations and seasons for species belonging to *Harveyi* clade with BOX- and ERIC-PCR (Canellas et al, 2021; Culot et al, 2021) and to investigate possibility for infection with species know as human pathogens we will determinate virulence factors for *tdh*, *trh* and possibly other genes.

Results

Our results showed that all observed parameters (culturable heterotrophic and total *Vibrio* bacteria in seawater, sediment, and bivalve tissues) were slightly higher in Lim Bay. Almost 75% of all culturable *Vibrio* bacteria belonged to *Splendidus* clade. Additionally, next most abundant clade was *Harveyi* clade (15%) and *Anguillarum* clade (1%). Species found belonging to *Harveyi* clade were *V. harveyi*, *V. campbellii*, *V. jasicida*, *V. parahaemolyticus*, *V. rotiferianus, V. diabolicus* and *V. alginolyticus*. Of all environmental parameters, we concluded that for abundance and diversity of *Vibrio* species most dominant parameter was temperature. We observed that trend is most visible in tissue samples, while for water and sediment was only observed for *Harveyi* clade. While during colder seasons *Splendidus* clade was more abundant, in warmer seasons *Harveyi* clade dominated. We determinate genomic fingerprinting for isolates belonging to *Harveyi* clade and found differences between species, locations and seasons and also reported presence of *trh* gene for virulence in *V. parahaemolyticus*. Results of antibiotic resistance show that more than 95% of all isolated bacteria are resistant to antibiotics and almost half of them (46.5%) are multi-resistant. Furthermore, most bacteria from all clades were resistant to vancomycin (95%). Next most abundant resistance was to ampicillin (45%). This study shows first report of presence of antibiotic resistance in bivalve aquaculture in Croatia and shows rising concern that with climate change and anthropogenic pressures which can and most likely will change environmental conditions, can negatively affect bivalve aquaculture both with bigger susceptibility of farmed bivalves to colonization by *Vibrio* pathogens and with more resistant *Vibrio* bacteria.

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Acknowledgements

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ANTIVIRAL EVALUATION OF A NOVEL PROPHYLACTIC TOOL FOR THE PREVENTION OF VIRAL DISEASES IN FISH

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Introduction

As the aquaculture industry grows, so does the emergence of pathogens and, with it, the need to seek new prophylactic measures. Up to now, the number of approved commercial vaccines is still small. Currently, these are vaccines based on attenuated viruses or based on recombinant proteins of viral antigens. Although they are safe, ensuring the stability and functionality of the protein and the type of administration hinder their effectiveness. Therefore, finding new formulations to maintain immunostimulatory or antigenic properties, as well as using oral administration as the most suitable method, remains a challenge. In this context, we have proposed an alternative based on the use of a new prophylactic design called "modular nanopellets (NPs)". It is the nanostructured recombinant protein of a viral antigen fragment coupled to an interferon gamma (IFNγ)-based cytokine effector domain to provide antiviral and adjuvant effects. Our working model consists of rainbow trout (Oncorhynchus mykiss) and two viruses relevant to salmonid aquaculture: VHSV and IPNV.

Materials and methods

At the *in vitro* level, the uptake of labelled NPs in RBCs, RTS11 and RTgutGC cells was quantified by flow cytometry and confocal microscopy. In addition, modulation of the expression of immune response genes in NPs-treated cells was assessed by RT-qPCR. *In vivo*, NPs were administered by intraperitoneal (i.p) injection into individual rainbow trout and the expression of immune response genes was assessed 48 hours post-vaccination. Also, blood sera were collected 30 days post-vaccination for titration of viral antigen specific antibodies and neutralizing antibodies.

Results

The results obtained showed a stimulation of the innate immune response, specifically the interferon pathway, by the modular nanopellets in RTS11, RTgutGC and rainbow trout RBCs. In addition, i.p injection of modular nanopellets demonstrated that fluorescent NPs could be detected in immune relevant tissues and stimulate the gene expression of immune genes in spleen, head kidney and rainbow trout RBCs. Also, VHSV and IPNV –specific antibodies were detected in the serum of individuals immunized by i.p injection. All these considerations provide a new perspective on the role and potential use of the modular nanopellets as a new antiviral prophylactic method alternative to the existing ones.

ANTIVIRAL ACTIVITY OF HIBISCUS (*Hibiscus sabdariffa*), GREEN TEA (*Camellia sinensis*), ECHINACEA (*Echinacea purpurea*) AND GRAPE SKIN (*Vitis vinifera*) EXTRACTS AGAINST THE VIRAL HEMORRHAGIC SEPTICEMIA VIRUS

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Introduction

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. Previously, the extracts from *Hibiscus sabdariffa, Camellia sinensis, Vitis vinifera* and *Echinacea purpurea* have shown their activity as immunostimulants or antivirals. In this study we evaluated the antiviral activity of these plant extracts against the viral hemorrhagic septicemia virus (VHSV).

Materials and Methods

The antiviral activity of the extracts was evaluated in EPC cell line. The viricidal capacity of the extracts as well as the antiviral activity before or during the course of infection were assessed. Viral loads were evaluated by means of FFC/mL. Cell fusion inhibition capacity assays were also performed to evaluate the viricidal capacity of the extracts. As well, we performed an in silico molecular docking study of the major compounds of the extracts in order to elucidate the most antiviral compounds.

Results

The results indicated that all extracts had antiviral activity, being one of them especially active and reducing the viral load up to a 50%. In addition, it was also observed that the most antiviral extract was able to reduce the fusion capacity of the virus and the major compound of this extract had the highest binding affinity to the viral proteins. In conclusion, the use of plant extracts as antivirals is presented as an alternative to mitigate viral diseases in aquaculture.

DESIGNING PRODUCTION LICENSES FOR SUSTAINABLE AQUACULTURE

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Introduction

Public regulation of a successful industry such as aquaculture needs to consider different concerns such as industry growth and development, but also environmental and societal sustainability. Governance systems are continuously challenged to respond in adequate manners to how aquaculture industry develops, especially in terms of how to best handle environmental challenges. We here investigate three different environmental regulations, all which aim to promote the development of more environmentally friendly production technologies to curb the negative environmental impacts of salmon aquaculture.

We ask two research questions:

1. How are production licenses designed to promote the development and ultimate use of environmental technology?

2. What are the main contributions and side-effects of these regulatory designs?

Methods

Based on earlier research, and an investigation of publicly available documents, including responses to public hearing processes, we study the design of three types of production licenses aiming to promote sustainable production of salmon aquaculture in Norway. The so-called "green" licenses, the "development" licenses, and the newly introduced "eco-technology" licenses.

Results

The table below provides an overview of some of the main characteristics and differences between the three types of licenses. The licenses differ in type of license, focus on risk, the requirements for technology development and implementation, area use, and permit requirements. Also, the possible impacts from these licenses will vary, partly due to their difference in volume. A total of 45 green licenses was awarded, while the number of development licenses is not yet finalized, it now shows 118 licenses (November 2021). The proposed availability of eco-technology licenses for the first year is limited to a maximum of 15 000 tons biomass (representing approximately 19 licenses a 780 mt). However, the eco-technology licenses are proposed to become a yearly allocation scheme so the magnitude of this scheme will much likely increase over time.

We find that the design of the licenses has changed considerable across the three schemes, but that the main contributions to regulation is to set the focus on environmental risks, and to give stimulus to technological innovation in fish farming. However, the side-effects are a large administrative burden and long-lasting award processes which at times has been characterized by lack of transparency and predictability.

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	Green licenses	Development licenses	Eco-technology licenses
Type of license	Ordinary (no time limit)	Special (time limited up to 15 years), can be converted to a ordinary license, at a fee	Special (proposed as time limited up to 20 years)
Environmental risks	Lice and escapes	Lice, escapes, area use	Lice, discharge, escapes, area use (inshore)
Environmental technology	Implementing new technology, and/or operational solutions aiming to reduce risk of escapes and sea lice. Technology should be tested, yet not commercialized	Developing + testing new technology (production unit) and new siting aiming to reduce challenges with environmental impact and area access. Technology must include significant innovations (compared to traditional technology).	Developing and commercializing technology that contributes to solving environmental and areal challenges. Aimed at various solutions for closed technology.
Area use	Ordinary sites	Ordinary sites + exposed/offshore and sheltered (closed technology)	Ordinary sites + sheltered
Investments	No investments requirements	Significant investments required	No investments requirements
Permit requirements	Requirement of use of technology and/or operational solutions (in line with the application), sea lice limit 0.25/0.1, for group A and B: one ordinary license must be "converted" to green license (following the same requirements) for each awarded green license.	Requirements for developing technology and the use of this during the testing period (in line with the application), no requirements for use of developed technology after conversion	Several indicators (zero dispersion of sea lice/egg, minimum 60 percent accumulation of discharge, and increased escape security), requirements to implement technology within 3 years
Sharing knowledge	Annual reports – available online (DoF)	Fact sheets, reports from developing phase, status reports from operational phase, and a final report at the end of project period – available online (DoF)	Plans for knowledge sharing are mentioned

ASTRAL'S OCEAN LITERACY FOR AQUACULTURE: INCREASING PUBLIC ACCEPTANCE AND AWARENESS OF AQUACULTURE IN THE ATLANTIC

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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) farming, 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 (fertilisers 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, SMEs, scientists, policymakers, social representatives, and other relevant stakeholders.

ASTRAL is committed to increasing the public acceptance and awareness of aquaculture by fostering public understanding of the value of aquaculture and especially IMTA, as a sustainable way to produce aquatic products. The project is also committed to reinforcing existing international efforts to advance acceptance and awareness about the sustainable exploitation of marine resources. ASTRAL works with a multilayer stakeholder's approach, therefore different Ocean Literacy activities have been designed around the project to ensure that the message reaches all the sectors of interest.

For example, targeting a wide public, specific campaigns in ocean literacy have been developed around the importance of safe and nutritious aquaculture seafood and addressing climate change resilience and ocean influence. During the World Ocean Day 2021, ASTRAL organised, in a coastal fishing community in Lagos (Nigeria), a variety of activities which included: 1) a clean-up campaign with the Makoko fishing community, 2) a thematic session about 'The Ocean and Livelihoods' and 3) training on 'Waste upcycling and women empowerment (Fig.1).

To engage a younger generation, during the Ideation Days 2022 at Terceira Island (Azores, Portugal), different activities were carried out specifically designed for secondary school students (Fig. 2). The activities were focused on science and innovation, including short thematic talks and a Bootcamp to promote in the audience the spirit of innovation and the creation of ideas. ASTRAL introduced the importance of Sustainable Food Production, explaining to the young audience concepts such as trophic level, carbon footprint, sustainability, and food security, and how IMTA contributes to sustainable food production.

Other examples of targeted Ocean Literacy campaigns are those addressing specific issues and engaging selected stakeholders, as in the case of women working in aquaculture. 70% of the global aquaculture workforce is female, nevertheless, the great majority of management of the production is undertaken by men. The lack of data on women in aquaculture and gender-oriented training is one reason why women are invisible in aquaculture policy. ASTRAL is working to reduce this unbalance by empowering women in the aquaculture sector by means of awareness and specific training activities designed to promote the role of women in aquaculture and increase its visibility in the sector. Examples of these activities are included in the All-Atlantic pledge campaign on Women's Empowerment in Aquaculture led by ASTRAL.

ASTRAL is a HORIZON 2020 project (GA 863034) financed under the Blue Growth programme. Further information: www.astral-project.eu/

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Fig. 1 World Ocean Day 2021: clean up with Makoko fishing community, thematic sessions, and waste upcycling activities. June 8th, (Lagos, Nigeria).

Fig. 2 Ideation Days 2022. February 28th (Terceira Island, Azores, Portugal)

ECOSYSTEM SERVICES BY INTEGRATED COASTAL AREA MULTI-TROPHIC AQUACULTURE: A STUDY WITH COMMERCIAL FISH FARMING AND BLUE MUSSELS

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Integrated Multi-Tropic Aquaculture (IMTA) has been promoted as a sustainable and practical alternative to monoculture that could help solve some ecological issues with intensified aquaculture in cage systems (Chopin et al. 2012, Custódio et al. 2020). IMTA involves the co-cultivation of tropically linked species where the lower trophic levels feed on the waste from the upper trophic levels, treating waste as a valuable resource utilised through natural synergistic interactions between the cultured species (Chopin et al. 2012, Kerrigan & Suckling 2018). Thus, the environmental impact is mitigated whilst economic value is added in secondary marketable products and reduced risk through product diversification (Barrington et al. 2009).

Blue mussels (*Mytilus edulis*) grown in surface waters have often been recommended in IMTA systems to extract particulate waste from finfish production (Cranford et al. 2013). However, it is debated if significant mitigation can be obtained by direct assimilation of fish farm waste (Sanz-Lazaro & Sanchez-Jerez 2017).

In all mitigation applications the local environment and the farming practice of the emitting species must be considered. Thus, a detailed analysis of the system and models of various possibilities must be conducted in order to ensure the best possible ecosystem benefits by the multi-trophic approach.

Salmon farming is the main aquaculture production in the Faroe Islands with most farms located in fjords. Recently the legislation has opened up for farming multiple species in a fjord and thus there is a potential for regional multi-trophic aquaculture.

In this study, the potential for multi-trophic aquaculture with salmon and blue mussels was modelled in a fjord with commercial fish farming. The best mitigation potential by blue mussels was investigated by modelling three spatial arrangements of blue mussels in the fjord with fish farming activity. Two fish farm/blue mussel IMTA conjunctions were investigated and a scenario where the blue mussels and fish farm were not directly connected by the particulate fish farm waste but still located in the same ecosystem.

The production at the commercial fish farm was analyzed to predict the emission of dissolved and particulate nutrients, and the dispersion of particulate waste around the fish farm was modelled. For the two IMTA scenarios the blue mussels were modelled next to the fish farm and below the fish farm and the direct uptake of particulate waste was traced (Fig. 1). In addition to the waste assimilation the nutrient uptake by the blue mussels through assimilation of microalgae was investigated in the three blue mussel farm setups. The hydrographic conditions in the fjord were measured and modelled and a two-year time series of the phytoplankton biomass and nutrient availability, as well as the growth potential of the endemic blue mussels, was investigated.

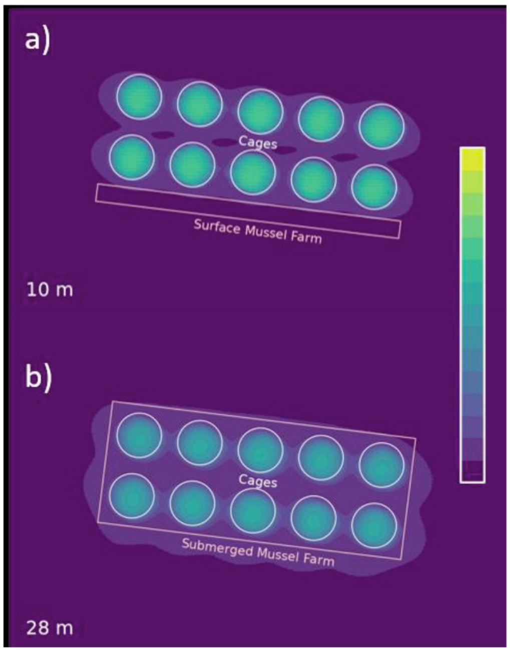

Figure 1. Concentration of waste particles at the base of the blue mussel farm at the surface at 10 m depth (a) and at the base of the submerged mussel farm at 28 m depth (b).From á Norði et al. (2022)

These data were used to investigate the potential removal of nitrogen by blue mussels and the mitigation potential by blue mussels was investigated in a coastal area management context, in order to predict the best mitigation potential considering the local production and spatial constraints in the fjord.

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Overturf

Utilizing RNA-seq, this study compared the transcriptomic responses of three improved strains (VSel, PSel, and CSel) of rainbow trout fry during acute stages of challenge with infectious hematopoietic necrosis virus (IHNV). The VSel strain has been selected for resistance against the specific strain of IHNV used in our challenge, PSel has undergone selection for utilization of plant-protein based feeds and previously has shown elevated non-specific disease resistance despite no disease related selection pressures, and the final strain, CSel, is a commercial strain that has been domesticated for several years but has not been selected for specific viral disease resistance. Following a 21-day IHNV challenge, Kaplan-Meier survival estimator curves and cumulative percent mortality (CPM) showed significant differences in IHNV resistance across strains: VSel - 19.3 ± 5.0%, PSel - 67. ± 3.03%, CSel - 94.6 ± 4.1% CPM. To evaluate acute responses to IHNV infection, whole blood, as well as samples from the kidney, liver, and intestine, were collected at 0, 4, 12, 24, and 48 hours post infection (hpi). Serum lysozyme activity, a marker of non-specific innate immunity, showed strain and temporal effects during the acute infection phase with PSel showing the highest activity at 0 and 48 hpi. Differential gene expression responses were detected, with varying degrees, in all tissues, both between strains, as well as across acute timepoints within strains. The VSel strain showed upregulation for a particular subset of viral recognition genes during early infection timepoints and rather limited upregulation of immune genes later, while maintaining and reactivating metabolic pathways. The CSel strain showed a downregulation of metabolic related genes and a limited upregulation of immune genes, while the PSel strain showed similar downregulation of metabolic genes during acute infection, yet when compared to the CSel strain, showed a more robust innate immune response. Evaluation of upregulated immune response genes, as well as interferon-related genes showed the PSel strain to have the greatest number of uniquely upregulated immune genes in both the kidney and intestine, with CSel and PSel showing a similar number of such genes upregulated in liver. A moderate number of immune response genes were shared between PSel and CSel in all tissues, though both PSel and VSel showed a high number of uniquely overexpressed immune response genes in the kidney, and PSel showed the highest number of uniquely upregulated interferon related genes in the intestine. Overall, the VSel response was unique from the CSel with very little overlap in activated immune responses. Findings from this study highlight the disparity in IHNV resistance among genetic strains of rainbow trout, while identifying molecular mechanisms underlying differences in disease phenotypes. Furthermore, our results on trout strains with distinct selection backgrounds yields comparative insights into the adaptive gains brought about by selection programs for pathogen-specific disease resistance, as well as the non-specific immune enhancement associated with selection for utilization of plant-based diets.

INSIGHTS INTO THE OVARIAN DEVELOPMENT OF THE MONANDRIC PROTOGYNOUS BALLAN WRASSE (*Labrus bergylta*)

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Introduction

The Ballan wrasse (*L. bergylta*) has become a popular choice of biological control against sea lice infection in salmon cages. Captive breeding and seed production technologies for Ballan wrasse are under continuous development and improvement to provide a sustainable source of cleaner fish and fulfill the increasing demand from salmon industry. Broodstock management has proved to be challenging due to the nature of their reproductive development, being a sequential hermaphrodite (Brooker et al, 2018). Current understanding of their reproductive biology is rather limited. For instance, it is still unclear if they are monandric or diandric protogynous hermaphrodite. The correlative endocrine changes during reproductive development in this species is also poorly studied. Thus, we aimed to investigate the onset of gonadal development in Ballan wrasse, specifically the timing and the progression of histological and endocrine features.

Materials and Methods

Ballan wrasse (n=103) were collected from a commercial hatchery in Scotland, UK. Samples ranged from 3-185 g in body weight, 5-19 cm in total length, and from 6 to 36 months of age. Gonads were subjected to standard histological processing and stained with H&E. Blood samples were also collected, plasma was separated and used for etheric extraction of steroids. Circulating levels of sex steroid hormones (17β-Estradiol (E_2), Testosterone (T) and 11Ketotestoterone (11KT)) were then quantified using commercial ELISA kits.

Results and Discussions

Histological examination of the gonads showed that 93% (96 out of 103) of the examined fish exhibited primary growth stage oocytes indicating that they are undergoing female maturation while 7% (7 out of 103) exclusively had oogonia (Table 1). Within a population of diandric protogynous species, some individuals directly undergo testicular development (i.e., primary males) while some mature first as female and then undergo sex reversal to attain male maturity (i.e., secondary males). In some species of reef fish where diandry was previously reported, the observed frequency of primary males in the population ranged from 13% to 30% (Lowe et al, 2021; Palma et al, 2019). In the present study, none of the 103 fish examined showed any signs of spermatogenesis indicating the absence of primary males. On the other hand, the presence of secondary males in this species has been well documented in the wild (Dipper & Pullin, 1979; Muncaster et al., 2013). Taken together, these observations strongly suggest that Ballan wrasse is a monandric protogynous hermaphrodite.

Entry of Ballan wrasse into primary oocyte growth was observed at around 23.9 ± 1.8 g and 10.7 ± 0.3 cm as evidenced by the presence of chromatin-nucleolar stage oocytes. Oocyte development progressed into perinucleolar stage and subsequently into cortical alveolar stage, which was correlated with increasing levels of circulating sex steroid hormones, E_2 and T. A significant increase in E_2 was particularly observed as fish developed cortical alveolar stage oocytes indicating the ensuing vitellogenesis, marked by estrogenic stimulation of hepatic vitellogenin biosynthesis (Lubzens et al, 2020). Gonadosomatic index also increased along with the observed ovarian development. Analysis of plasma 11KT showed basal values consistent through various stages, which further support the idea that all fish examined were developing towards female maturity.

In summary, our findings suggest that Ballan wrasse is a monandric protogynous hermaphrodite. Moreover, this is the first study to describe the histological features and correlative steroid hormone profiles during the onset of gonadal development in Ballan wrasse. Ongoing analysis is being conducted to determine the expression levels of reproductive genes in the gonad as well as the circulating levels of gonadotropins. Information generated from this study provides a basis for the development of effective broodstock management and reproductive technologies for Ballan wrasse.

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Table 1. Morphometry of Ballan wrasse at different stages of ovarian development[1].

	Og	Cn	Pn	CA
BW (g)	11.7 ± 1.1	23.9 ± 1.8	100.5 ± 14.2	146.6 ± 8.8
TL (cm)	9.2 ± 0.7	10.7 ± 0.3	15.9 ± 0.9	18.0 ± 0.3
GSI (%)	0.06 ± 0.02	0.10 ± 0.02	0.28 ± 0.03	0.31 ± 0.02
HSI (%)	0.76 ± 0.30	1.24 ± 0.12	1.43 ± 0.12	1.25 ± 0.11

[1] Og – Oogonial stage; Cr - Chromatin-nucleolar stage; Pn - Perinucleolar stage; CA - Cortical Alveolar stage

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HEART RATE AND ACCELERATION LOGGING DURING SWIM-FITNESS AND STRESS CHALLENGE TESTS IN YELLOWTAIL KINGFISH (*Seriola lalandi*) AND RELATION WITH SWIMMING SPEED, OXYGEN CONSUMPTION AND LOCOMOTORY BEHAVIOR

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Introduction

Yellowtail kingfish is a fast-growing athlete that requires sufficient water flow over the gills to meet the oxygen demands. It is successfully being farmed in marine RAS but energy economy under these conditions poses challenging metabolic issues. Moreover, swim-training at optimal speed enhances body growth, lowers feed conversion ratio, increases cardiac output capacity (Palstra et al., 2015) and represents a promising approach for application at the farm.

In this study, we attempted to gain more insight into the energy economy by investigating heart rate and acceleration, as determined by application of Starr-Oddi loggers, at increasing swimming speeds during a swim-fitness test, and during an induced stress challenge test by repeatedly lowering of water levels (Svendsen, 2021). Oxygen consumption was measured and locomotory behavior (including tailbeats and respiration frequencies) monitored by high speed camera recordings during the swim-fitness test and parameters were related to heart rate and acceleration. Ultrasound was used as additional determinator of heart rate, although under anesthesia.

Materials and methods

Fish – Yellowtail kingfish from the Kingfish Zeeland farm were transported to the animal experimental facilities of CARUS in Wageningen. N= 16 fish were used for surgical implantation of the loggers and N= 14 fish were controls without a logger. Implanted fish was allowed to recover for 10 days before experiments were started.

Loggers – DST milli-HRT ACT (Starr-Oddi, Gardabaer, Iceland) loggers were implanted (Fig 1AB). Heart rate was derived from the Electrocardiogram (ECG) signal at 200 Hz recorded every 10 min as beats per minute (bpm) and acceleration as average external acceleration value (AvEA in milli-g-force, which is an average of 600 measurements over 1 minute) recorded at 10Hz.

Swim-fitness test – Fish were swum in series of three in three Blazka type swim tunnels (van den Thillart et al., 2004; Fig. 1C). Fish were kept at rest and then swimming at incremental swimming speeds of 0.2, 0.4, 0.6, 0.8 and 1.0 m s^{-1} for one hour each. The first N= 8 implanted fish were immediately dissected for the loggers after the swim test, the next N= 8 implanted fish were used for the stress challenge test.

Stress challenge test and cortisol – During the stress challenge, N= 8 implanted fish were kept with the controls in a tank and then exposed to four conditions: reducing water level and (1) filling up immediately, (2) after 1 min, (3) after 5 min, and (4) after 5 min with chasing the fish (Fig. 1DE).

Ultrasound – An Esaote MyLabFive Vet ultrasonography unit (Esaote Europe BV, Maastricht, the Netherlands) with a 18MHz LA435 ultrasound transducer and Pulsed Wave and color (CFM) Doppler was used as second methodology for determining heart rate, under anesthesia after swim fitness and stress challenge tests.

Results and discussion

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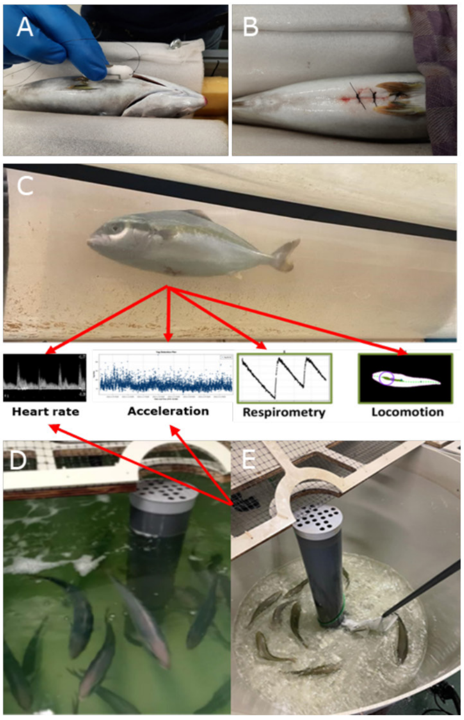

Figure 1. Overview of the experimental approach, showing (A) the positioning where the logger was implanted, (B) the stitch to anchor the logger and two additional stitches to close the wound, (C) a swimming yellowtail kingfish with implanted logger in the swim-tunnel where, additional to heart rate and acceleration, also oxygen consumption and locomotory behaviour were monitored, (D) the fish still at high water level and (E) at low water level inducing stress.

When fish were subjected to the swim-fitness test, implanted fish measured 33.2 ± 2.2 cm total length (TL) and weighed 652 ± 152 g body weight (BW), control fish measured 34.7 ± 2.2 cm TL and weighed 729 ± 122 g BW. Optimal swimming speed (Uopt) for implanted fish was 0.84 ± 0.06 m s^{-1} at a Cost of Transport (CoT) of 169 ± 32 mg kg^{-1} km^{-1}, for control fish Uopt was 0.82 ± 0.11 m $s^{-1}$and CoT was 178 ± 40 mg kg^{-1} km^{-1}. There was no impact of the logger on the oxygen consumption during swimming.

Base line heart rate of fish in the tank was 110 ± 19 bpm and accelerations were AvEA= 12 ± 4 milli-g. Heart rates of fish in the swim tunnel were higher but stable between 126 to 139 bpm up to swimming at 0.6 m s^{-1} and then increased up to 155 ± 2 bpm at 0.8 m s^{-1} and 162 ± 7 bpm at 1.0 m s^{-1}. Accelerations increased linearly with swimming speed from AvEA= 11 ± 1 to 26 ± 4 milli-g. The first, second and third lowering of the water level increased heart rate up to 138-144 bpm and accelerations were up to AvEA= 26 milli-g during the first but decreased to AvEA= 19 and 15 milli-g during the second and third stress induction. The fourth lowering of the water level that included the chasing caused the highest heart rates at 186 bpm with accelerations of AvEA= 44 milli-g. When anaesthetized, the eight implanted fish that were subjected to the stress test had a heart rate of 47 ± 11 bpm as determined by ultrasound.

An integrative data analyses will be presented also including the locomotory behavior results which are currently being analyzed.

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OUT-OF-SEASON REPRODUCTION OF TWO DIFFERENT PIKEPERCH (*Sander lucioperca*) STRAINS: EVALUATION OF BROODSTOCK REPRODUCTIVE PERFORMACE AND EARLY LIFE STAGE DEVELOPMENT OF THEIR OFFSPRING

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Introduction

The commercial farming of pikeperch (*Sander lucioperca*) has improved over the last years. Due to the development of different applied protocol, several production bottlenecks, such as controlled reproduction and constant supply of juveniles, have been tackled. As a result, the production of pikeperch in controlled conditions is slowly but constantly increasing in Europe. Despite the substantial progress observed over the last decade, pikeperch is still considered an emerging aquaculture fish species, facing different challenges throughout the production cycle. One of these challenges is the lack of information on broodstock management. While some research has focused on the nutritional requirement of the broodstock, and effect of rearing environment, there is a lack of information about the performance of different strains under similar controlled conditions. Therefore, the aim of this study was to evaluate the reproductive performance of two different pikeperch broodstock strains and the development of their offspring during the early life stage, under similar controlled conditions, in recirculating aquaculture systems (RAS).

Material and methods

In 2018, RAS-grown pikeperch (MBW= 0.5 ± 0.1 kg) coming from two different origins, “Inagro19”, origin A, and “Germany19”, origin B, were tagged and placed in a RAS-broodstock climate room (mixed tank). For two years, the fish were exposed to Inagro’s photo-thermal protocol for gonadal cycle induction, but they were not propagated. In 2021, during the third photo-thermal regime, oocytes from all the females were periodically sampled to determine oocyte growth and for the selection of females for the artificial reproduction. Sixty fish in total (15 females and 15 males from each strain; MBW = 2.1 ± 0.3 kg), were artificially propagated according to the protocol described by Żarski et al. (2019). The reproduction performance of both strains was evaluated by assessing different parameters during the propagation such as oocyte maturation kinetics, egg quality, fertilization, survival and hatching rate among others.

After hatching, larvae from each strain were randomly distributed in eight 220-L tanks connected to a RAS (4 tanks per strain) with an initial density of 110 larvae per liter. For twenty-nine days (29 DPH) larvae were reared according to Inagros’ hatchery protocol. Briefly, water temperature was kept at 16 ºC, initial live feed (AF + EG enriched artemia) followed by weaning to dry feed and a photoperiod of 16L:8D. During this period, growth performance, swim bladder inflation (SBI), survival rate and malformations were evaluated. Data was statistically analyzed for significant differences (α = 0.05) between strains.

Table 1. Reproductive performance of pikeperch females from two different strains (Inagro19 and Germany19) during artificial propagation

Parameters	Inagro19	Germany19	*P*
Ovulated females (%)	100	93	0.99
Fertilized females (%)	67	50	0.46
PGSI (%)	10.5 ± 2.6	12.2 ± 3.1	0.19
fertilization rate (1-cell stage)	84.2 ± 5.1	82.4 ± 6.04	0.69
Hatching rate %	69 ± 29	81 ± 15	0.21

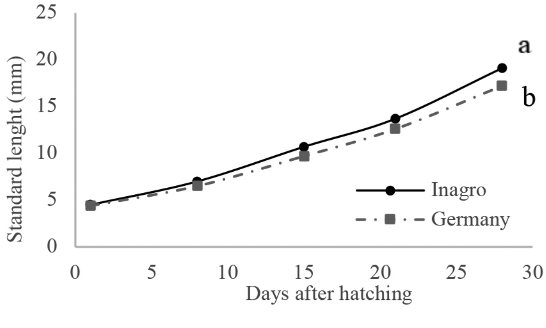

Figure 1. Standard length of pikeperch larvae from two different strains (Inagro19 and Germany19) reared from 29 day under similar condition

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Results

In general, the reproductive performance of both strains was similar. The oocyte growth during the photo-thermal protocol was slightly higher in Inagro19 compared to Germany19, however, no significant differences were found ($P>0.05$) between strains. Similar results were found in the parameters evaluated during propagation (Table 1). On the other hand, differences in oocyte maturation kinetics were found between strains. Inagro19 females reacted faster to the hormonal treatment, reaching oocyte stage 3-4 after 72-hour hormonal injection, while oocytes of Germany19 females were at stage 1-2, with some exceptions, after 72-hour hormonal injection.

Regarding growth performance of the offspring, at 29 DPH larvae from Inagro19 strain were significantly larger than larvae from Germany19 strain (Fig 1). Similar results were observed in mean body weight, with larvae from Inagro19 being 28% heavier compared to those from Germany19.

Discussion

More information regarding the origin of the fish is being collected in order to better understand the results obtained. This information will be added and discussed during the presentation.

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ASSESSMENT OF GROWTH, BODY COMPOSITION AND NUTRIENT PERFORMANCES OF JUVENILES AND PRE-ADULT EUROPEAN SEA BASS (*Dicentrarchus labrax*) FARMED IN RED SEA CONDITIONS WITH COMMERCIAL DIETS

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Introduction

The European seabass is among the most farmed species in the Mediterranean Sea for its environmental characteristics that make it suitable to thrive between 5° to 28°C and from brackish to seawater conditions. The aquaculture of European seabass in southern latitudes can greatly benefit from all-year round productions due to the lack of cold seasons and the possibility to obtain optimal feed conversion ratio (FCR) and specific grow rate (SGR) at temperatures of 26°C-30°C.

With its long coastline, different climatic conditions and good quality water the Kingdom of Saudi Arabia is particularly advantaged in developing marine aquaculture. The northern part of the Red Sea looks the most promising for the farming of Mediterranean species due to its average temperatures between 27°C to 29°C despite higher salinity of 42 g L^{-1}. The scope of the current research was to determine the growth performance and nutrient retention of juvenile and pre-adult European seabass fed with commercially available marine feeds and to compare the performances against the results obtainable in the Mediterranean Sea.

Materials and Methods

For the first trial (Run 1) juvenile fish of 126.6 g fish were stocked in 1 m^3 tanks at a density of 4.2 kg m^{-3}. For the second trial (Run 2) pre-adult fish of 314.0 g fish were stocked in same capacity tanks at a density of 5.9 kg m^{-3}. Fish were reared at a salinity of 42 g L^{-1} and average temperature of respectively 27.0°C (Run 1) and 25.9°C (pre-adult) under a photoperiod of 12:12 hours. The duration of the trials were respectively 84 days (trial 1) and 80 days (trial 2).
Fish were fed at libitum daily twice a day four types of locally available commercial feed with the following levels of crude proteins and crude fat (CP/CF): 47/14 (Diet 1), 48/14 (Diet 2), 47/12 (Diet 3), 42/20 (Diet 4).

All data was analyzed using one-way ANOVA followed by Fisher Least Significant Difference (LSD) test.

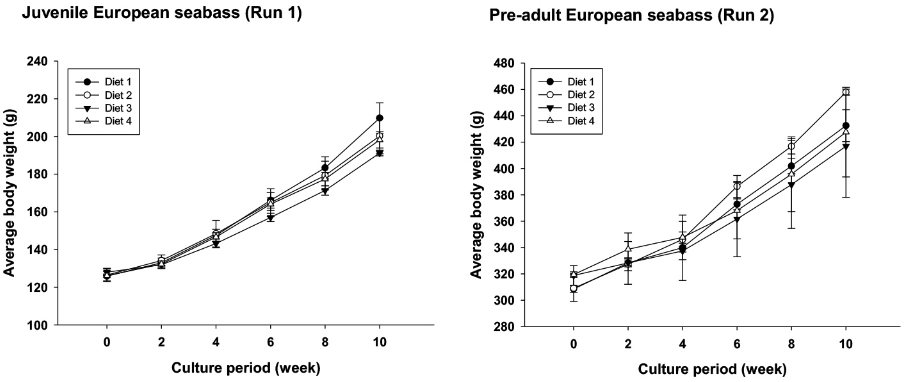

Fig. 1. Initial and final average body weight of juvenile and pre-adult European seabass fed the different commercial diets. Values represent means ± S.E.M. (n=3).

(Continued on next page)

Results

For the first trial the final body weight ranged between 191 g and 210 g, but no significant differences were noted among the diets, however the SGR was highest ($P < 0.05$) in Diet 1 (0.65) and lowest in Diet 3 (0.51). No statistical differences were noted among Diets for FCR (1.73 - 1.76) with the exception of Diet 3 (1.97) ($P < 0.05$).

For the second trial fish reached the final body mass of 417-458 g, but no statistical differences were observed. No differences were also seen for SGR, with values ranging between 0.35 and 0.52. Similarly the FCR showed no statistical differences, with values being respectively 1.7, 1.5, 2.5 and 1.9 among the Diets 1-4.

Discussion

The current study showed that the growth performances of the E. seabass are comparable to other researches carried out at lower temperatures or salinities, where juvenile fish could attain SGRs and FCRs of respectively 0.5-0.7 and 1.30-1.8 (Tibaldi et al., 2015; Güroy et al., 2006; Eroldoğan et al., 2000).

The pre-adult fish had similar or better results than other studies, where fish could achieve SGRs of 0.3-0.6 and FCRs of 1.8-2.2 (Mladineo et al., 2010).
The proximate analysis of the whole body of the fish did not show any differences among treatments for both crude protein, lipid, ash, with the only exception of higher ($P < 0.05$) gross energy in Diet 2 (12.32 MJ kg^{-1})

The protein retention efficiency showed values of 11.8 - 16.4, much lower than those measured by Lanari and D'Agaro (2005) (24.5% - 25.0%), which may have been caused by lower levels of digestibility of the feed ingredients or lower levels of essential amino acids (EAA).

Conclusions

The growth of European seabass in Red Sea condition is comparable to other researches. Further improvements in growth and FCR can be easily achieved through feeds that make use of more digestible ingredients and increased levels of EAAs.

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DIFFERENTIALLY EXRESSED SMALL RNAS IN THREE DIFFERENT OVARIAN MATURATION STAGES OF HATCHERY-REARED GREATER AMBERJACK *Seriola dumerili*

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Introduction

The first steps in greater amberjack domestication have been completed and the species can be currently produced under captivity. However, hatchery-reared fish have difficulties in achieving reproductive maturation under captivity and exhibit smaller gonads, lower levels of sex steroids and extensive oocyte atresia compared to wild individuals (Zupa et al., 2017). The aim of the present study was to explore the role of epigenetics, and more specifically of small RNAs, in ovarian development of hatchery-reared greater amberjack. With this aim, immature, maturing (late vitellogenesis) and spent ovaries of hatchery-reared greater amberjack were collected, and the differential expression of small RNAs in the three different ovarian maturation stages was studied.

Materials and Methods

Gonads used in the present study were collected from hatchery-reared greater amberjack at the end of the first year of life (immature, Papadaki et al., 2021) and at two different samplings at Argosaronikos fish farm, Salamina, Greece in June (maturing) and July 2015 (spent, Zupa et al., 2017). Four female fish from each group were killed on ice, the gonads were excised, one piece was preserved in a formaldehyde solution for histology and another piece in RNAlater for RNA extraction. After RNA extraction and quality check, micro RNA (miRNA) libraries were generated, sequenced and differential expression analysis was conducted.

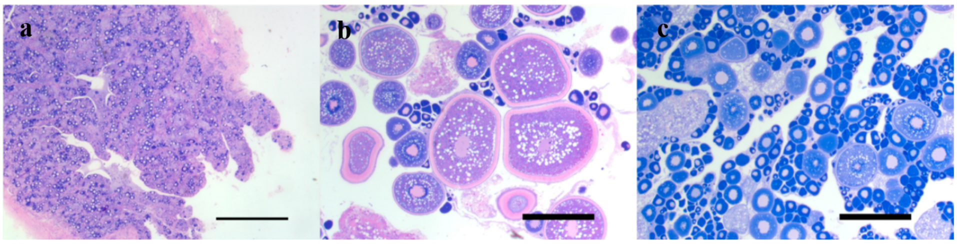

Figure 1. (a) Immature ovaries, mostly containing oogonia and primary oocytes, b) maturing ovaries, exhibiting late vitellogenic oocytes and c) spent ovaries with primary and atretic oocytes of hatchery-reared greater amberjack.

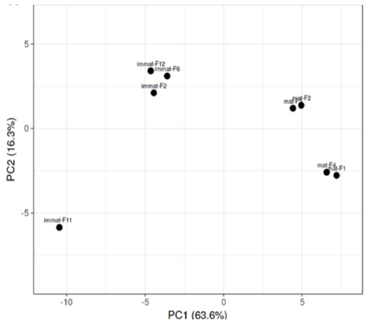

Figure 2. Principal component analysis of immature (IF2, IF6, IF11 and IF12) and spent (MF1, MF2, MF3 and MF4) greater amberjack ovaries. X and Y axis show principal component 1 and 2 that explain 63.6%, and 16.3% of the proportion of variance, respectively.

(Continued on next page)

Results

Immature individuals exhibited gonads, containing mostly oogonia and primary oocytes (Fig.1a), maturing individuals exhibited vitellogenic oocytes (Fig.1b) and spent individuals showed mostly primary and atretic oocytes in their gonads (Fig.1c).

Regarding sequencing results, the average number of reads for each sample after quality and adaptor trimming was 12 million reads. Between immature and spent females, two distinct groups were revealed by Principal Component Analysis (PCA, Fig. 2), with principal component 1 and 2 explaining 63.6% and 16.3 % of the total variance, respectively.

Discussion

Hatchery-reared greater amberjack can reach vitellogenesis under captivity but exhibit elevated atresia and low sex steroid levels. In the present study, the differential expression of small RNAs and the relevance with the ovarian maturation stages in hatchery-reared greater amberjack are discussed.

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EFFECT OF FEEDING FREQUENCY ON PRODUCTIVE INDICES IN OPTIMUM OXYGEN SATURATION LEVELS FOR SEA BREAM (*Sparus aurata*) AND SEA BASS (*Dicentrarchus labrax*)

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Introduction

Along with the quality and type of feed in a fish farm, the formation of an optimal and most effective nutritional protocol is influenced by many factors. It is a fact that the feeding frequency can affect the amount of feed consumed, the behavior of the fish and their growth performance. Optimal feeding frequency reduces fish farm production costs and prevents water quality degradation caused by overfeeding (Daudpota *et al*., 2016). This study aimed to investigate the effect of feeding frequency on productive indices in sea bass and bream in optimum oxygen saturation levels.

Materials and Methods

Two feeding frequency trials were conducted at the Aqualabs facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (HCMR) for sea bream and sea bass. Thirty-five individuals were used for both experiments. The trials were carried out in closed recirculation systems (RAS), each consisting of 500L capacity tanks under fully controlled oxygen, light and water flow conditions. In each closed circuit, the optimal level of oxygen saturation in the rearing water for each species was applied, as determined by previous experiments. Specifically, for sea bream, the oxygen range was 60-80%, while for sea bass was 80-100%. Different feeding regimes were applied in each RAS. Three treatment groups were fed to satiation at one of three frequencies (one, two and four meals per day) for 90 days. Three replicates were performed for each feeding regime. The feedings frequencies were determined by the rate and degree of gastric evacuation (stomach and intestine), from previous experiments (Table 1). Three gastric evacuation points were chosen at 25% (four feedings per day), 50% (two feedings per day), and 75% (one feeding per day).

Table 1. Feeding frequency and feeding times for sea bream and sea bass

Feedings	1st – four feedings	2nd – two feedings	3rd – one feeding
	Daily feeding hours		
1^{o}	8:00	8:00	8:00
2^{o}	12:00	16:00	
3^{o}	16:00		
4^{o}	20:00		

Table 2. Growth/ somatometric indices for sea bream under three different feeding frequencies at 60-80% oxygen saturation (mean ± SD)

	1 feeding	2 feedings	4 feedings
Initial weight (gr)	75.8 ± 0.52 ^{a}	74.9 ± 0.86 ^{a}	76.0 ± 0.52 ^{a}
Final weight (gr)	350.1 ± 6.47 ^{a}	362.6 ± 1.82 ^{a}	365.7 ± 6.63 ^{a}
CVmb	3.20 ± 0.06 ^{a}	0.87 ± 0.00 ^{b}	3.14 ± 0.06 ^{a}
Daily growth rate (G%)	4.02 ± 0.12 ^{a}	4.27 ± 0.08 ^{a}	4.23 ± 0.06 ^{a}
SGR%	1.70 ± 0.03 ^{a}	1.75 ± 0.02 ^{a}	1.74 ± 0.01 ^{a}
FCR	1.31 ± 0.04 ^{a}	1.35 ± 0.00 ^{a}	1.38 ± 0.02 ^{a}
DFC	1.89 ± 0.01 ^{a}	1.96 ± 0.01 ^{b}	2.00 ± 0.03 ^{b}
Weight gain (WG%)	361.8 ± 10.9 ^{a}	375.3 ± 9.1 ^{a}	380.9 ± 5.8 ^{a}

Table 3. Growth/ somatometric indices for sea bass under three different feeding frequencies at 60-80% oxygen saturation (mean ± SD)

	1 feeding	2 feedings	4 feedings
Initial weight (gr)	78.97 ± 0.35 ^{a}	78.60 ± 0.32 ^{a}	78.20 ± 0.37 ^{a}
Final weight (gr)	178.4 ± 5.51 ^{a}	214.7 ± 4.42 ^{b}	221.6 ± 2.77 ^{b}
CVmb	5.36 ± 0.16 ^{a}	3.58 ± 0.07 ^{b}	2.16 ± 0.03 ^{c}
Daily growth rate (G%)	1.52 ± 0.12 ^{a}	2.09 ± 0.12 ^{b}	2.21 ± 0.05 ^{b}
SGR%	0.98 ± 0.03 ^{a}	1.21 ± 0.03 ^{b}	1.25 ± 0.01 ^{b}
FCR	1.30 ± 0.03 ^{a}	1.22 ± 0.03 ^{a}	1.22 ± 0.02 ^{a}
DFC	1.16 ± 0.01 ^{a}	1.33 ± 0.01 ^{b}	1.42 ± 0.01 ^{c}
Weight gain (WG%)	125.8 ± 5.98 ^{a}	173.1 ± 5.94 ^{b}	183.4 ± 2.62 ^{b}

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Results and Discussion

Growth indices for sea bream are shown in Table 2, whereas for sea bass are in Table 3. The results for sea bass showed that the recommended number of feedings is two per day (at 8:00 and 16:00), with 8-hour feeding interval. In both sea bass and sea bream, a reduction in the amount of feed consumed in the first meal was observed for the groups that had repeated meals within the day (Kikuchi et al., 2006). However, no improvement in growth was observed when the frequency of feeding was increased from two to four per day for both species. The FCR remained constant in both species. Despite the higher feed consumption for the group of four feedings compared to the others for sea bass, this is not reflected in the growth performance of the species. In the case of sea bream, no statistically significant difference was seen even in the amount of feed consumed between the groups for the duration of the experiment. Fish fed fewer times per day showed greater motility during feeding, as the opportunities to receive feed were limited per individual, thus developing an anticipatory food activity (AFA). According to Chen and Purser (2001) this developed ability of fish helps to increase feed intake and feeding efficiency.

Acknowledgments

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GENETIC CHARACTERIZATION OF AN ENDANGERED SPECIES OF SICILIAN INLAND WATERS: THE MEDITERRANEAN TROUT (*Salmo cettii*)

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Mediterranean trout (*Salmo cetti*), is endemic species belonging to *Salmo trutta* group which is widespread exclusively in Sardinia, Sicily on a few areas of the Italian Tyrrhenian side. Following repopulation carried out with Atlantic trout, its presence on the Italian territory has undergone a strong contraction due to hybridization and introgression effects. At the moment, *Salmo cettii* is on the IUCN Red List, rated as Critically Endangered (CR). The selection of pure breeders with high genetic variability and the storage of their sperm in a cryobank become important tools to make the management of the conservation of endangered species more efficient. Recent pieces of evidence indicated the presence of a specific haplotype in the Iblei Mountains of Sicily suggesting the presence of some conserved areas with the endemic populations (Segherloo et al., 2021). The objective of the work was to genetically characterize a population of Mediterranean trout (*Salmo cettii*) caught in different waterways of the Iblei Mountains of Sicily in order to verify its level of introgression with the allochthonous Northern Atlantic haplotypes and evaluate the possibility to use this population as a precious resource for future restocking plans.

Materials and methods

57 wild Mediterranean fish were caught from different waterways of Sicily Iblei Mountains, in order to sample their genetic material. For the comparative genetic analysis, the samples from 41 *S. trutta* of Northern Atlantic lineage were collected (29 from Morgex Val d'Aosta and 12 trout from Fiumelatte –(Como) hatcheries). Genetic analyses were performed using: i) 16 autosomic STR microsatellite; ii) SDY E2S, a sex marker; iii) the Locus LDH-C1; iv) the mtDNA D-loop. Allele frequency-based analyses was detected by GenAlEx program (Peakall and Smouse 2012). Genetic structure and gene flow were analyzed by STRUCTURE 2.3.4 (Pritchard et al.2000). The combination of the results deriving from the analysis of the LDH-C1 and the mitochondrial D-loop were performed by the hybridization index (Penserini et al. 2006).

Results

The analysis of the SDY-ES2 locus revealed that, out of the total Sicily Mediterranean trout, 42 were males and 15 females. The expected and observed heterozygosity (He: 0,630 and Ho: 0,627, respectively) was quite low, as was the actual number of alleles (Ne: 2,70). A deviation from a Hardy-Weinberg equilibrium was found for 9 out of 16 loci ($p < 0.05$). STRUCTURE analysis clearly revealed the presence of 2 distinct populations, with few misassignments in the Northern Atlantic populations.

The Locus D-loop mtDNA analysis showed that the Sicily Mediterranean population 100% matched with the haplotype sequence present in the database for *Salmo cetti* (GenBank MW251439.1, Segherloo et al., 2021), except for 3 individuals. The screening of the LDH-C1 Locus revealed the presence of 3 individuals with allele LDH-C1 * 90. The hybridization index indicated the 87% of individuals belonging to Class VI (zero potential of hybridization), 6% to Class V and only 8% to Class II (high potential of hybridization) (Pensierini et al., 2006).

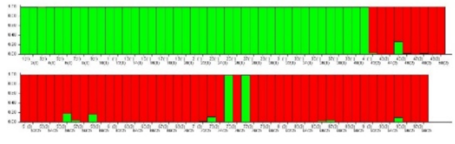

Figure 1. Barplots of individual, STR-based admixture as obtained through the Bayesian clustering analysis in STRUCTURE

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Discussion and Conclusion

The results obtained in this study indicated that the Mediterranean trout population sampled in some waterways of Sicily Iblei Mountains showed a very low level of introgression with the Norther Atlantic lineage, despite the numerous releases of the allochthonous individuals occurred in the past. Probably, the extreme conditions of the waterways and the permanence to which they are forced make difficult the survival of the North Atlantic haplotype and the relative hybrids. At the same time, the geographic isolation of individuals, due to the characteristics of the waterways, represent a limit to the genetic variability of the population, which was found quite low. The genetic results obtained in this preliminary study suggests a potential use of the Sicily Iblei Mountains trout population as a precious resource for planning native fish restocking activities.

Acknowledgements

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REFINING ORGANIC AQUAFEEDS FOR RAINBOW TROUT, GILTHEAD SEA BREAM AND MEAGRE: IMPLICATION ON GROWTH, FISH HEALTH AND PRODUCT QUALITY

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Introduction

The new Strategic Guidelines for the sustainable development of EU aquaculture adopted by the Commission in spring 2021 intend to further promote organic aquaculture as strategy to meet consumer demand for diversified high quality food produced in a way that respects the environment and ensures animal welfare. EU organic regulation (889/2008 and 848/2018) established that only fish meal derived from fish trimmings and from whole fish caught in fisheries certified as sustainable together with vegetable raw materials derived from organic plants can be used as the main protein sources for organic aquafeed. The difficult to reach certified ingredients, together with the high production costs, are the main problems of low production of organic EU fish. The needs for additional types of raw materials and reconsidered the sourcing of plant feed ingredients has been recognised as necessary to promote EU organic aquaculture. The aim of this study was to test the effects of certified organic novel raw materials (organic pea protein, organic yeast, organic seaweed) on growth, fish welfare and products quality of rainbow trout, gilthead sea bream and meagre.

Materials and Method

Three feeding trials (one for each species) were performed in order to replace 60, 50 and 45% fishmeal trimming (FM) in rainbow trout (RT), sea bream (SB) and meagre (M) respectively, using increasing level of organic pea protein (PP), organic seaweeds and organic yeast. Three experimental diets for rainbow trout (control, RT-C; PP 10%, RT-PP10; PP 21%, RT-PP21) and sea bream (control, SB-C; PP 8%, SB-PP8; PP 19%, SB-PP19) and four diets for meagre (control, M-C; PP 7%, M-PP7; PP 18%, M-PP18 and M-PP27) were formulated using organic raw materials according to EU organic regulation. Diets were tested in triplicated fish groups (initial weight, RT: 63g; SB:146g; M: 33g) to satiation over 60 days for RT and SB and during 85 days for M.

At the end of the trials specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR), somatometric indexes (hepatosomatic index, HSI; viscerosomatic index, VSI) and nutritional indices (protein efficiency ratio, PER; gross protein efficiency, GPE) were performed for all species. In addition, fish quality parameters (fillet and liver proximate and fatty acids composition and sensory quality by trained panellists) were performed in SB and RT. In M fish health was assessed by plasma biochemistry and gut microbiome by next-generation sequencing. Differences among treatments were considered significant at $P < 0.05$.

Results

At the end of trials, no significant differences in growth (SGR, FI, FCR) were observed in M, while SB-PP8, SB-PP19 and RT-PP21 showed lower SGR in comparison to the respective control diets. FCR was lower in RT-PP10 than RT-C and RT-PP21. Concerning welfare parameters glucose was higher in animals fed M-C and lower in M-PP27. Total protein and albumin showed higher values in animal fed M-PP18 and lower values in M-C. Albumin/globulin was higher in animals fed M-PP27. In RT and SB no significant differences were observed in HSI and fillet yield while VSI was higher in RT-PP21 than the other treatments. Fillet in both SB and RT showed a higher protein but lower lipid content in PP21. In RT, fillet showed a higher content of DHA and N3-PUFA in the fish fed RT-PP21 in comparison to the other diets. Similarly, DHA and N3-PUFA were higher in SB-PP21 than SB-C. Some similarities in sensory characteristics were observed when comparing SB and RT samples fed with organic feeds containing different proportions of PP. Effects were observed in odour, appearance, and textural sensory attributes. In particular, flesh of both species had a less intense ammonia odour, a lower quantity of exudate and was firmer but also less crumbly and juicy. In addition, the inclusion of PP significantly increased the bitter flavour perception in SB and RT, even though the effect was not significant for RT samples.

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Discussion

This study highlighted the possibility to partially replace FM using novel organic vegetable raw materials (pea protein, seaweed and yeast) in rainbow trout, sea bream and meagre. The inclusion of organic pea protein at low levels in combination with organic seaweed and/or organic yeast did not produce any effect on growth and/or composition of the fish, however a high inclusion of pea protein produced a lower growth in trout, but not in sea bream and meagre. The inclusion of the organic raw materials tested induced a higher accumulation of N-3 PUFA in the fillet of trout and sea bream. Similarly, the results of the sensory characterisation of the fillet also showed a positive effect of pea protein inclusion on odour, appearance and textual sensory attribute leading to a higher product quality.

Acknowledgement

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

PILOT STUDY OF BEHAVIORAL ANALYSIS IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) INFECTED WITH *Betanodavirus*

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Introduction

The study of animal behavior is a sector that is attracting increasing interest from the scientific community, also thanks to the new technologies available to researchers. In recent years, fish have also entered this type of study, despite the difficulties of observation due to the aquatic medium.

Materials and methods

In this pilot study, the critical points in setting up studies of this kind in an experimental aquarium were evaluated and the applicability of the system to observe the behavior of sea bass (*Dicentrarchus labrax*) infected and not with *Betanodavirus* was evaluated by daily video recording of 2 tanks and subsequent analysis using dedicated software (EthoVision XT, Noldus Information Technology, Wageningen, The Netherlands).

Results

The results highlighted several critical issues in the experimental set-up, both at the hardware level (lighting, aerosol, humidity) and at the software level (reflections, shadows, individual tracking). Concerning behavioral analysis, the infected fish had a lower movement speed and distance covered and a higher state of inactivity than the control group. In addition, the infected fish showed greater dispersion within the tank than the control group.

Conclusions

These results highlight how the progression of the disease under examination influences the behavior of fish, beyond the clinical signs, providing interesting and useful predictive tools for the improvement of animal welfare and the management of diseases.

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DIETARY CHITIN LEVEL AFFECTS NUTRIENT DIGESTIBILITY AND PLASMA ENZYMES AND METABOLITES IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Chitin-containing invertebrates, in particular insects, are a promising protein- and mineral-rich resource, characterized by a low environmental footprint and increasing availability. For the above-mentioned reasons, they have recently become attractive as a potential aquafeed ingredient[1–3]. The use of this innovative ingredient, as a partial substitute for conventional protein sources, has been tested for different aquacultured species[4–7]. The observed results are promising both for growth performance and diet digestibility, even if impairment in growth performance and nutrient availability due to the presence of chitin has been reported with contrasting results according to finfish species[8,9]. Therefore, the effect of the inclusion of 0%, 1,5%, 3%, and 4,5% chitin from prawn shells in semi-purified diets for rainbow trout (*Oncorhynchus mykiss*) on growth, feed utilization, nutrient digestibility, and plasma parameters has been investigated.

Materials and methods

Four dietary treatments have been formulated to contain 48% crude protein and 16% crude lipid. All diets were added Celite® as an inert marker before being cold extruded and dried into 3 mm pellets at the facilities of the University of Udine. The diets tested were the control diet (0% chitin), and three diets where graded levels of chitin (Merck, cod. C9213) 1,5, 3,0 and 4,5% replaced cellulose. These levels simulate the dietary inclusion levels of 15, 30 and 50% of a commercial partially defatted insect meal from black soldier fly (Protix**®)**. The fish used in the study were purchased from a commercial fish farm and were adapted to the experimental conditions 2 weeks prior to the onset of the experiment. Almost constant temperature (13±0,7**°C) and water conditions were assured during the trial. One hundred and eighty rainbow trout (**27,4±2,38g) were randomly allotted among five tank units each including three vessels singularly stocked with 15 fish and fitted with a settling column for faeces recovery[10]. Fish were hand-fed one daily meal (9:00) to a fixed ration (1% live weight). After the meal, feed eventually refused were promptly removed. Per each diet, faeces were collected over two independent 15-day periods. At the end of the trial, fish were group-weighed after 6 hrs fasting and 6 fish per treatment were euthanized with an overdose of anaesthetic (150 mg/L MS 222) and blood samples were immediately collected from the caudal vein for plasma enzymes and metabolites analysis. Freeze-dried faecal samples were analysed for dry matter, ash, nitrogen, lipid, and acid insoluble ash as outlined by National Research Council (2011)[11]. Apparent digestibility coefficients (ADCs) of dry matter, crude protein (CP), organic matter and lipid were calculated[12].

Results

Fish promptly accepted all the diets. Feed intake, growth performance, feed conversion and specific growth ratios of rainbow trout were calculated over 10 weeks. Dietary chitin did not affect weight (56,87±2,05g), feed utilization (1,01±0,07) or specific growth rate (1,08±0,11%) till 3% inclusion level. The higher inclusion level of chitin (4,5%) significantly reduced both final weight (42,48±1,05g) and growth rate (0,73±0,06%) and feed utilization (1,66±0,25) ($P<0,05$) and this was first a consequence of the lower absolute feed intake.

The *in vivo* digestibility coefficient for lipid and protein showed a significant decrease when the chitin was included at the highest percentage ($P<0,05$). The apparent digestibility coefficient (ADC) for the control diet resulted in 87,68 % for protein, 91,99 % for lipid and 69,97% for organic matter. The ADC of test diets resulted 86,76%, 83,91% and 68,30% for protein; 93,60%, 93,39%, and 81,54 for lipid; 69,89%, 65,47% and 57,74% for organic matter, respectively for diet CHI1,5, CHI3, and CHI4,5.

Most of the plasma enzymes and metabolites related to nutritional (cholesterol, glucose, triglycerides, protein and albumin) and physiological conditions (lactate dehydrogenase (LDH), aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT)) were not affected by feeding chitin up to 3% included diets. In particular, fish fed CHI0, CHI1,5 and CHI3 diet, showed the highest values for cholesterol (197±43,6, 216,9±40,7 and 226,5±36,5 mg/dl), triglycerides (168,31±55, 179,1±42,1, 170,54±41,3 mg/dl), protein (2,23±0,2, 2,32±0,2, 2,09±0,2 g/dl) and albumin (1,75±0,2, 1,63±0,2 1,62±0,2 g/dl respectively). On the other hand, fish fed the highest dietary chitin inclusion level showed the lowest value for cholesterol (122,05±36,3 mg/dl), triglycerides (84,9±28,5 mg/dl), protein (1,7±0,2 g/dl) and albumin (1,29±0,2 g/dl) also ($P<0,05$ for cholesterol and triglycerides; $P<0,001$ for protein and albumin).

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Discussion

Recent years have seen an increasing interest in insect meals as a sustainable and nutritious innovative ingredients to be included in aquafeed [13]. More recently, their health-promoting potential is also going to be explored. This experimental trial showed that a dietary chitin level of up to 3%, does not affect the zootechnical performance, the nutrient digestibility and the plasma enzymes and metabolites in rainbow trout. On the contrary, 4,5% chitin (roughly simulating a diet including 500g/kg of defatted *Hermetia illucens* meal) negatively affected growth performance, feed utilization, nutrients digestibility and plasma parameters.

Conclusion

Dietary chitin inclusion up to 3% is well tolerated in rainbow trout while higher inclusion (4,5%) level significantly affected growth, feed utilization, nutrient digestibility and plasma enzymes and metabolites levels. This suggests that insect meal can only partially replace conventional protein sources in diets for rainbow trout and insect protein derivatives could be considered.

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THE ROLE OF ALGAL BIOFILMS AS CUE FOR SETTLEMENT AND EARLY GROWTH IN THE SEA CUCUMBER *Holothuria tubulosa*

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Introduction

The larval settlement is one of the trickiest stages during the development of many benthic marine invertebrates due to the complex processes of interaction between biotic and abiotic factors operating at different spatial and temporal scales. Such a process interests the larval cycle of *Holothuroidea* species that is characterized by three main stages, a feeding planktonic auricularia, a non-feeding planktonic doliolaria, followed by the settlement into pentactula, which feeds on the substrate (Rakaj et al., 2018). Such a critical step is also reflected in the rearing of sea cucumbers, where the low settlement success and the low survival rate of post-settlers represent the bottleneck to the feasibility of commercial-scale production. Several cues have been used to stimulate the metamorphosis of sea cucumbers, including natural biofilms, the addition of seagrasses extract, and the conditioning of settlement plates with diatoms biofilms (Agudo 2006; Rakaj et al., 2018).

In order to improve the information on the role of algal biofilm in the induction of larval metamorphosis, we investigate the effect of different biofilms on the settlement success of the doliolaria of *Holothuria tubulosa* and we also provide information on the growth of juveniles reared for two years in a pilot-scale hatchery.

Materials and methods

The metamorphosis of doliolaria larvae of *H. tubulosa* was tested with three treatments: the diatom *Amphora spp.*; the green algae *Ulvella lens* and the Control (no biofilm). The experimental design considered multiple chamber plates (6 chambers each plate; 4 replicates each treatment) with a volume of 3 ml for each chamber. Before the trials, the plates were conditioned with the respective biofilms.

The larvae used in this study were bred in three 150-L tanks (1 larva ml^{-1}) and were fed with the pabulum made of a mix of the microalgae *Chaetoceros calcitrans*, *Isochrysis galbana*, and *Tetraselmis suecica*. The larvae reached the doliolaria stage after 25 days post-fertilization. From this batch of larvae, 72 doliolaria were transferred individually in the trial chambers that were kept at 24°C with a photoperiod of 12h light and dark. The progress of the settlement was followed under a stereomicroscope, every five days, by observing the morphological changes from doliolaria to pentactula. When the second posterior podium appeared, the specimens were considered juveniles. The settlement success was calculated as the ratio of the number of settlers (pentactula and juveniles) to the number of initial larvae, expressed in percentage. The settlement performance was tested at the end of the experiment after 35 days. Prior to the one-way ANOVA ($P < 0.05$), Cochran's C-test ($P>0.05$) was used to check the assumption of the homogeneity of variances. *Post-hoc* multiple comparisons were performed using the Tukey HSD test. The remaining doliolaria larvae of the batch described above (ca. 60000), were transferred in three 500-L tanks provided with *Amphora spp.* biofilms. For the following two months, larvae were fed with diatoms *ad libitum*. After this period, larvae were fed three times a week with spirulina (*Arthrospira platensis*) mixed with fine sand and a commercial feeding powder (at a feeding rate of 3% of juveniles' weight). Every three months sea cucumbers were visually counted for survival rate determination and a subsample of 10 juveniles was randomly collected from each tank, measured (total length in mm), and weighed (total wet weight in g) for growth parameters determination.

Results

The settlement success at the end of the experiment was about 21%±12.5se with *Amphora spp.*, and 54%±12.5se with *U. lens*, which was the only treatment significantly different from the Control. Moreover, the treatment *U. lens* showed the highest post-settler survival, with 25% of juveniles, while in the treatment *Amphora spp.* only 4% of the larvae reached the same stage. The percentage of swimming doliolaria decreased with the progression of the settlement, except in the Control plates, where at the end of the experiment, 62% of the doliolaria were still present.

The larvae transferred in the pilot-scale hatchery showed survival of about 1% after seven months post-fertilization, with the production of ca. 600 juveniles, of which, 50% survived after 15 months post-fertilization and showed a mean weight of 6.8g ±3.0sd, a mean length of 41mm± 10.4sd.

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Discussion and conclusion

Sea cucumbers larvae bred in captivity are generally induced to settle on natural biofilms obtained by immersing the plates in diatoms cultures for a few days or adding an extract of *Sargassum sp*. or seagrass leaves (Agudo 2006, Rakaj et al., 2018). Our results showed that all the plates conditioned with algal biofilm allowed the settlement of the doliolaria and its metamorphosis into pentactula. The best settlement performance was achieved with *U. lens*, which allowed the metamorphosis of the 54% of the larvae into pentactula, confirming the high performance of this alga in fostering the settlement of echinoderms larvae (Hannon et al., 2017). It is particularly interesting to observe that larvae that survived in the Control plates were still in the doliolaria stage for the whole duration of the experiment. This fact indicates that the presence of some sort of cue is a necessary condition to proceed with the metamorphosis of *H. tubulosa* and that larvae are able to delay metamorphosis when no substrate is available. These results could be explained by the *Desperate Larva Hypothesis*, revised to be applicable to the non-feeding stage of the larval cycle (Botello and Krug, 2006). According to this hypothesis as the non-feeding stage continues, larvae became "desperate" to settle and the level of selectivity towards the substrata decreases. Indeed, the lower number of juveniles in the treatment with *Amphora spp*. (4%) can indicate that while the cue seems to be enough to induce the settlement, *Amphora spp*. alone, might not be able to ensure high survival of the post-settlers and their growth.

The results provide practical applications increasing the efficiency of the current rearing protocols for sea cucumbers. In fact, we reported the first record of growth-out of juveniles at a pilot-scale hatchery for *H. tubulosa*.

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OPTIMIZATION OF SEA CUCUMBER BREEDING PROTOCOL: NEW TRIGGERS FOR THE SPAWNING IN *Holothuria tubulosa*

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Introduction

The increase of uncontrolled harvesting led to the overexploitation of many holothurians species, including the Mediterranean sea cucumber *Holothuria tubulosa* (Gmelin 1791). Commercial-scale aquaculture can represent the alternative to the exploitation of wild populations and such technologies have been provided for the most valuable species (i.e. *Holothuria scabra*) (Agudo, 2006). Experimental attempts to breed Mediterranean sea cucumbers were recently reported by different authors (Domínguez-Godino and González-Wangüemert, 2018; Rakaj *et al.*, 2018). However, the adaptation of the technologies and the breeding protocols used with tropical species to the Mediterranean ones presents some criticisms, including the low effectiveness of the stimuli used to collect the gametes. To optimize the current breeding protocols, we explored new different triggers to stimulate the spawning in *H. tubulosa* broodstock.

Materials and methods

A total of 200 adults in a reproductive stage were collected by SCUBA diving in the Gulf of Teulada (Italy, Sardinian Sea) in August 2020. After collection, each broodstock was placed in a 3-L plastic bag and transported to the experimental hatchery in cooling boxes to avoid thermal stress. Once in the hatchery, specimens were divided into four acclimation tanks (350L), at a reference temperature of 25°C, with the natural light regime, for 48h prior to the experiment.

To trigger the gametes emission from broodstock, the following set of stimuli was considered: thermal stressors (alone or combined with air exposure), the addition of conspecific sperms or feeding excess and bubbling (Agudo 2006; Ferranti et al., 2018; Rakaj et al., 2018). Each treatment was replicated in 6 independent tanks (15-L), containing 3 sea cucumbers each, and all treatments lasted 180 minutes. Except for the thermal stressors, the water temperature in the treatment tanks was set at a reference value of 25° C, i.e. the same temperature as the acclimation tanks. At the end of the exposure, specimens were moved into the spawning tanks (one for each replica), filled with seawater (25°C). During the treatments and the next 9h, the behavior of sea cucumbers was observed to detect whether they assumed the pre-spawning position (Ocaña and Tocino 2005) and/or they have spawned. When spawning occurred, specimens were isolated in separate tanks.

The gametes obtained with the most effective trigger were used to perform the hatching rate trials in 2-L tanks (three replicates), using the following densities of the fertilized eggs: 4, 8, 25, 50, 100, 200 eggs ml^{-1}. The embryos were kept in the dark, with static water at 24°C. After 24h, the hatching rate was volumetrically estimated by collecting 1 ml subsample (n=3) from each replicate and the number of swimming larvae in the gastrula stage was counted.

One-way analysis of variance was performed to test differences in the spawning trials and the hatching rate trials. Data expressed in percentage were arcsin transformed ($x' = \arcsin\sqrt{x}$). Prior to performing the ANOVA, we tested for homogeneity of variance with Cochran's test. The *post-hoc* Tukey HSD test was applied to test significant effects between each treatment and the Control and/or among treatments ($P < 0.05$).

Results

All the specimens used in the experiment showed no signs of stress (e.g. evisceration) and no mortality occurred. The triggered specimens showed the pre-spawning behavior, followed by the emission of the gametes. The best trigger was the bubbling, with the emission of 61%±25.1 of specimens, followed by the feeding excess (22%±17.2) using diatoms. Both these treatments were different from the control, where no specimens spawned. Few specimens also spawned in with the two types of thermal stressors (6 %±13.6). The eggs released in the treatment bubbling were estimated in 5.4±1.5sd million oocytes, and this batch was used to assess the hatching rate of the fertilized eggs. The hatching rate was negatively affected by the increase of the densities and drastically dropped at the highest density, with only the 3%±0.6 of hatched eggs.

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Discussion and conclusion

Among the investigated treatments, bubbling was the most effective stimulus, leading to the emission of more than 60% of specimens. This stimulus, despite having never been investigated with echinoderms, is the most common method used for the reproduction of other broadcast spawners marine invertebrates (Ferranti et al., 2018). This treatment acted by increasing the water turbulence in the tanks using vigorous air insufflations, possibly recreating the turbulence of the wave motion, whereas in all the other treatments the water in the tanks was static. Indeed, the water movement can also be the cause of the spawning of sea cucumbers during the transportation from the wild to the laboratory, which can be enough to trigger the specimens (Agudo, 2006; Rakaj et al., 2018). The sensitivity of sea cucumbers to the increase of water turbulence as a stimulus to trigger the gametes' release might be an adaptive strategy for these broadcast spawners. In fact, moderate water turbulence can enhance the dispersion of the gametes, increasing the probability of their encounter and the subsequent fertilization (Gaylord 2008), thus fostering the reproductive success.

In conclusion, the trigger "bubbling" can provide a considerable amount of vital gametes, both oocytes and sperms. These can be fertilized and hatched into the gastrula stage, which, in the vision of a commercial-scale hatchery, can improve sea cucumber production.

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ENVIRONMENTALLY SUSTAINABLE SOLUTIONS FOR PRODUCTIVE RECOVERY OF OSTREA EDULIS

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Introduction

Shellfish culture is traditionally supported by the collection of wild juveniles. For Ostrea edulis, however, this practice is inefficient given the scarcity of biological material available in the lagoon. The productive recovery of native species requires strengthening the production capacity of existing hatcheries. However, the production increases should not adversely affect the environment and ecosystem in which these are grown, especially when they are located in fragile areas but of high cultural, landscape and natural value. The objective of the present study is to evaluate new technologies and solutions to improve the productivity and environmental sustainability of hatcheries for pre-growing of Ostrea e. for their productive recovery.

Experimental

The study can be divided into three main steps:
Optimization of reproduction condition through digitalization.
Synthesis of feed for oysters from macroalgae
Biofilters from waste (shells)

Results

Optimization of reproduction condition through digitalization.
Long-term tests were performed to verify the stability of the response of the sensors placed in the closed-circuit tanks of the hatchery. The purpose of the tests was to verify the accuracy of the data provided by the sensors (pH, salinity, dissolved oxygen, temperature, chlorophyll, ammonium, and nitrate) and to establish the timing required for their maintenance (cleaning and calibration) in order to ensure their optimal operation and the reliability of the results obtained. The survival rate of specimens divided by size class of Ostrea edulis in tanks controlled with sensor and non-sensor systems were monitored.

Synthesis of feed for oysters from macroalgae
Average composition of the main nutritional constituents of algae (Ulva lactuca and Gracilaria) undergoes some significant variation in relation to seasonality. The two seaweed species studied differ significantly in ash and lipid content. Ulva lactuca shows a higher content of both. Since the uses and health benefits of unsaturated lipids contained in many marine animal and plant species are known, the lipid profile of seaweeds was studied. In addition, the suitability of microalgae extracts for use as feed ingredient for oysters. In particular, the organic and inorganic micropollutants were determined. The algae-based material was encapsulated using a mixture of lipids and alginate to produce small droplets. The encapsulated feed was tested on bivalve mollusc larvae. The results obtained showed that the optimal growth rate of the larvae was achieved by using a mixture of encapsulated feed and 50% phytoplankton.

Biofilters from waste (shells)
A wastewater filter system was installed at Naturedulis (Figure 1), consisting of a cylinder filled with a mixture of adsorbent materials obtained from shells.The efficiency of the proposed system in the relevant environment was tested. Water samples were taken before and after the filter treatment, which are subjected to chemical analysis. The data obtained shows that the filter system removes more than 50% of the heavy metal in water.

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APPLYING THE CONCEPT OF TOLERANCE LANDSCAPES TO BUILD SCENARIOS OF CLIMATE IMPACT FOR CULTURED BIVALVES

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Introduction

With ongoing climate change, extreme climatic events, such as heatwaves, are predicted to increase in frequency, intensity, and duration. The thermal tolerance of organisms is also dictated by a combination of exposure intensity and duration.: long exposures at temperatures lower than the critical temperature (CT_{max}) can still be deleterious. Since bivalve farming has been recently advocated as a 'restorative aquaculture' practice with some climate mitigation potential, it would be of key to understand how these species are faring under current climate change scenarios.

Methods

Tolerance landscape theory (Rezende et al., 2014) where was used in this study to predict the effects of heatwaves. Two species were chosen: the Mediterranean blue mussel *Mytilus galloprovincialis* and the manilla clam *Ruditapes philippinarum:* the former is farmed in the water column while the latter on the seabed, and together they account for more than 95% of all farmed shellfish in Italy. A tolerance model for *R. philippinarum* was already developed by Bertolini & Pastres (2021) and the same procedure was followed to build a model for *M. galloprovincialis,* based on literature data. The main goal of this study was to investigate how heat waves could affect the mortality of these species, considering the different dynamics of water and sediment temperature in a shallow water body. To this aim, scenarios of both water and sediment temperature in the Lagoon of Venice, where both species are farmed, were constructed for the decades 2020-2100. The water temperature scenarios were defined by assuming an additive structure of the time series. Average hourly values were obtained by interpolating monthly values provided by OGS in the framework of the "Venezia 2021" research project. Scenarios of water temperature until 2100 were based on estimates provided by the SHYFEM model, a deterministic model using a 2D grid over the ~~whole~~ water body, which assumes the RCP 8.5 climate scenario. These monthly values were interpolated to hourly values to form a general trend. Daily oscillations were estimated based on the spectral analysis of a comprehensive set of bi-hourly water temperature time series collected in the Lagoon of Venice from 2008 to 2018 (SAMANET network).

Sediment temperature scenarios were estimated based on a cross-correlation model between time series of water and sediment temperature data collected in 2020/21, The temperatures below ground were lower in summer and higher in winter, thus two separate linear models were applied to predict the sediment temperature based on the daily mean water temperature. For each year , 2019-2100, the highest value of consecutive hours spent above each temperature threshold starting from 25°C at 0.1°C until the maximum temperature recorded (30°C) was used to compare against the thresholds identified by the tolerance landscapes (water temperatures for *M. galloprovincialis* and sediment temperature for *R. philippinarum*).

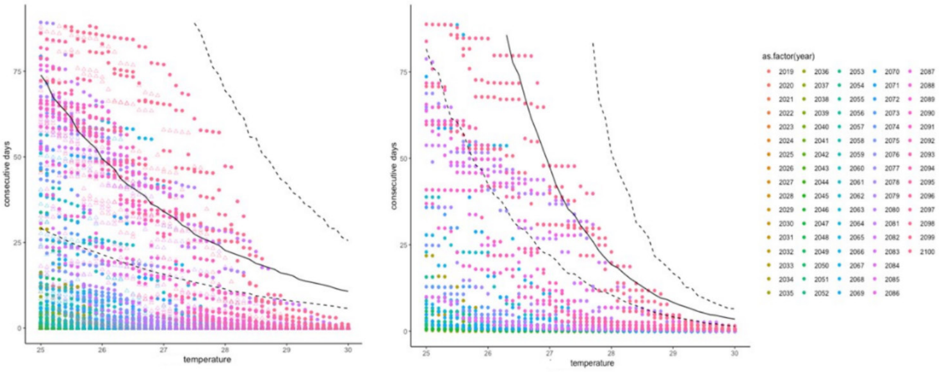

Fig.1 Maximum value of consecutive days above each temperature (left: sediment, right: water) compared to tolerance landscape (full line, dotted lines represent 95% CI) for *R.philippinarum* (left) and *M. galloprovincialis* (right).

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Results & Discussion

The values of CT_{max} and z for *M. galloprovincialis* were 39.7 °C (± 1.7) and -2.64 (±0.4) °C, while the values for *R. philippinarum* in Bertolini & Pastres (2021) were 54.5 °C (± 2.3) and - 5.72 °C (± 0.66), both consistent with bivalve values from Rezende et al. (2014). The values suggest that blue mussels have a lower CT_{max} but can sustain high temperatures for longer compared to clams, due to a lower value of z.

When scenarios of water temperature and sediment temperatures are compared to the thresholds (Fig.1) it is observable that temperatures for both species are reaching and surpassing the threshold within the end of the century, for *R. philippinarum* entering the lower confidence boundary by the 2070s (with some outlier years in previous decades) while for *M. galloprovincialis* towards the end of the 2070s. It is also noticeable that the surpassing of the threshold is happening in greater proportion at the lower temperatures, in particular for the earlier years.

Conclusions

This study shows that, towards the end of the century, both of these commercially important species will face detrimental conditions, in which temperatures regularly exceed their thresholds. Findings also demonstrate that the duration of moderate heat waves can be a more severe threat to clam survival than short, extreme heat waves. However, since temperature forecasts were developed using the RCP 8.5 climate scenario, these results resemble the worst-case scenario.

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VISIBLE EFFECTS OF STRESS ON FISH

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Introduction

An increasing number of tools is being developed for assessing the effects of stress on fish. But most of these methods are invasive and often require the sampling of blood or other tissues for the analyses. Consequently, using visual procedures for assessing the effects of stress on fish may be advantageous since they don`t require the handling of the animals. In our study, the chronic effects of stress on common carp (*Cyprinus carpio* L.) were investigated.

Material and methods

The fish were kept in groups of six in separate 90l aquaria. Animals were treated with one of 6 different stressors every day for 7 and 28 days in order to avoid habituation to the stressors, and were challenged on the sampling days with air exposure as an additional stressor. Control groups were reared in parallel and either left untreated during the entire experiment or challenged with air exposure only on the sampling days. In week 1 and 4 of the experiment, the fish behaviour was monitored for 20 min each day using GoPro Hero9 cameras, and after 28 days of treatment also images of the body have been taken in order to analyze the body coloration.

Results and discussion

Our results show that the analysis of fish behaviour and body coloration may be useful as indicators of stress. The behaviour of fish clearly is influenced by exposure to different stressors. However, assessment of behavioural differences is time-consuming and automatization strongly depends on the quality of the video material. Thus, taking pictures of the fish may be an easier way to assess effects of stress since automatic analyses of images is more efficient.

Acklowledgement

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EFFECT OF DENSITY AND MIXED CULTURE OF LARGEMOUTH BASS (*Micropterus salmoides*) WITH PIKEPERCH (*Sander lucioperca*) ON GROWTH, SURVIVAL AND FEED CONSERVATION RATE IN INTENSIVE CULTURE

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Introduction

The intensive freshwater aquaculture using the recirculating aquaculture systems (RAS) is new approach for getting more efficient, stable, profitable and high-quality marketable fish production which is currently mainly supported by the traditional pond and water through culture methods (Martins et al. 2010)i.e., to produce food while sustaining natural resources is achieved only when production systems with a minimum ecological impact are used. Recirculating aquaculture systems (RASs. The using of polyculture in the RAS with similar requirements on environment and with different behavior, swimming and feeding activity, offers great potential to more effectively use capacity of this kind of aquaculture (Thomas et al. 2020). The aim of this study was to optimize the intensive largemouth bass culture in the RAS and to monitor the impact of the initial density on the efficiency of its production, as well as to compare the impact of the biculture of largemouth bass and pikeperch on their production efficiency with the monoculture of both species.

Material and methods

The first 140days experiment assessed the effect of initial fish density in three levels: low: 23kg.m^{-3}; medium: 35 kg.m^{-3}; high: 46 kg.m^{-3}. In total, 3303 pcs. of experimental fish [with total length (TL) = 217.8–225.2 ± 18.2–19.7 mm and body weight (BW) = 139.9–157.1 ± 37.2–43.6 g] were stocked in nine tanks connected to large scale experimental RAS. After 8 hours of light, the fish were kept for another 4 hours at a reduced light intensity (until 7:00 pm) of 70–80 lux in order to offer the largemouth bass an optimal light period and support fish sufficiently by feed and maintain all routines in RAS. Experimental fish of all groups in all tanks were fed 50% by hand and 50% by belt feeder from 8:00 am to 7:00 pm with actual calculated daily feeding rate (DFR) at 28day interval. At the beginning of the experiment, DFR was set at 1.5% of fish biomass and DFR was reduced to 0.75% of fish biomass from the middle of the experiment (from 72nd day of this experiment). Fish were fed commercial floating feed R-2 Europa F 15 (Skretting, Norway) with a size of 2.0 mm (at the beginning of the experiment) to 3 mm (at the end of the experiment). Such pelleted feed was collected, and all non-consumed pellets were counted.

The second experiment tested the effect of mixed culture of largemouth bass and pikeperch compared to monoculture of both species during 60days intensive aquaculture. Same aged fingerlings of largemouth bass (TL = 103.8 ± 3.5 mm; W = 12.5 ± 2.5 g) and pikeperch (TL = 121.7 ± 5.5 mm; W = 12.2 ± 1.9 g), which were produced by a combination pond and RAS culture according Policar et al. (2013). In total, 700 fish were stocked in each tank (in monoculture only one species a in biculture both species with ratio 50:50) with initial density 1.17 fish and 14.5–14.8 g per liter. Before the experiment, all experimental fish of both species were size sorted and only fish with minimum size differences (9.0–16.0 g) in individual body weight were selected for this study. At the end of the experiment, all the fish were weighted again, and the frequency of the fish at same 1 g interval with final size difference (11.1–75.0 g) was again calculated.

Results

In the first 140days experiment, all three densities provided same high specific growth rate (SGR = 0.22–0.24 %.d^{-1}). Feed conversion ratio (FCR) was the lowest for low (1.39 ± 0.21 g.g^{-1}), the highest (1.61 ± 0.08 g.g^{-1}) for medium density. High density did not have any different FCR (1.50 ± 0.17 g.g^{-1}) compared to other tested densities. Analogous results as for SGR were also obtained for survival rate (97–100%). The second 60days experiment tested the effect and comparison groups of largemouth bass and pikeperch (*Sander lucioperca*) within monoculture and biculture on production efficiency. The higher FC was evaluated higher in both groups (monoculture and biculture) of largemouth bass (1.09) compared to groups of pikeperch (0.74–0.78). SGR were the lowest for both groups (monoculture and biculture) of largemouth bass (1.20–1.28%.d^{-1}). In contrast, the highest SGR was in the group of biculture pikeperch (1.88 %.d^{-1}). The similar results such as SGR were also assessed for FCR, where the higher FCR were in both groups of largemouth bass (1.44–1.48 g.g^{-1}) than in the biculture of pikeperch (0.73 g.g^{-1}). All four tested groups had a high survival rate (98.61–99.99 %) (Fig. 1).

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Discussion

The optimal fish density as high as possible is very important fact of successful intensive aquaculture in many species (Ronald et al. 2014). Higher density of intensively cultured juvenile Eurasian perch (0.5–15 g) from 400 to 10 000 fish.m^{-3} can increase growth rate and decrease growth heterogeneity. This positive correlation between fish density and growth rate was valid until 10–16 g perch juveniles. In bigger perch juveniles up to 16 grams, increasing density from 20 to 60 kg.m-3 decreased growth rate (Mélard et al., 1996). This positive relationship can have effect on higher survival rate of cultured fish. The results show that fish survival rate during the experiment was significantly lower for the pikeperch than for the largemouth bass. There may be several reasons for these differences in survival of both tested species, which have the slightly different physiological processes, their tolerance and adaptation to conditions of intensive farming (Bell 2013)but its operation is restricted by ecological and genetic constraints. The cost of natural selection expresses the limited capacity of a population to sustain the load of mortality or sterility required for effective selection. Genostasis expresses the lack of variation that prevents many populations from adapting to stress. While the role of relative fitness in adaptation is well understood, evolutionary rescue emphasizes the need to recognize explicitly the importance of absolute fitness. Permanent adaptation requires a range of genetic variation in absolute fitness that is broad enough to provide a few extreme types capable of sustained growth under a stress that would cause extinction if they were not present. This principle implies that population size is an important determinant of rescue. The overall number of individuals exposed to selection will be greater when the population declines gradually under a constant stress, or is progressively challenged by gradually increasing stress. In gradually deteriorating environments, survival at lethal stress may be procured by prior adaptation to sublethal stress through genetic correlation. Neither the standing genetic variation of small populations nor the mutation supply of large populations, however, may be sufficient to provide evolutionary rescue for most populations. © 2012 The Author(s.

Acknowledgements

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INTEGRATING SEA CUCUMBERS IN FISH AQUACULTURE WASTE RECYCLING: A MODEL OF SUSTAINABLE BLUE GROWTH

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Introduction

Fin-fish aquaculture negatively affects the benthic community's health through the depositions of large amounts of fecal matter, leftover food and catabolites on the sea bed. In this context, the polyculture of species with different ecological role and feeding behaviors in the same integrated multi-trophic aquaculture (IMTA) system is an innovative culture approach to minimize the environmental impact caused by the strong growth of the fin-fish industry. Among extractive species, deposit-feeding sea cucumbers are the most promising to diversify and maximize production, reducing at the same time the organic load of impacted areas. Sea cucumbers, indeed, are able to ingest sediment and organic material, playing an important role in cycling and energy flow in benthic ecosystems. Moreover, these marine invertebrates are economically attractive species being considered luxury seafood, especially in Asian countries. Laboratory and pilot-scale studies have already suggested Mediterrenan sea cucumber species as promising candidates for IMTA applications (Neofitou et al., 2019). However, a scarcity of information is available about their culture under open-water farms (Tolon et al., 2017; Bastien Sadoul et al., 2022; Cutajar et al., 2022). This study, therefore, aimed to provide baseline information on the survival and growth performances of Mediterrenean sea cucumber species, *Holothuria tubulosa* and *Holothuria polii*, when cultured at three different rearing densities under a productive sea bream farm.

Materials and Methods

During the Summer of 2020, a IMTA experiment was carried out at "Piscicoltura del Golfo di Gaeta Soc. Agricola a.r.l.", which is a commercial fin-fish farm located in the central Tirrenean Sea (Italy). Sub-adults of *H. tubulosa* and *H. polii* were collected at Torre Astura (Italy) and starved for 48 h in lantern nets. After starvation, for each species, individuals were weighted and placed in seabed cages beneath the fin-fish cages at three rearing densities: Low, Medium and High, respectively of 500-1000 g m^{-2}, 1000-2000 g m^{-2}, 2000-3000 g m^{-2}. The suitability of *H. tubulosa* and *H. polii* to use fin-fish waste as the sole food source was evaluated in terms of Survival and Relative Weight Gain (RWG %) over a four-month experiment.

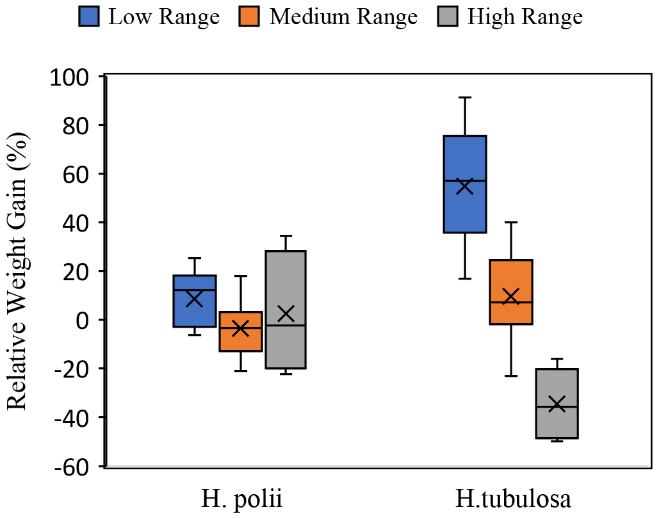

Figura 1. Relative Weight Gain (%) of H. tubulosa and H. polii cultured at three stocking density range (Low, 500-1000 g m^{-2}; Medium, 1000-2000 g m^{-2}; High, 2000-3000 g m^{-2}) under commercial fin-fish farm for three months.

(Continued on next page)

Results

At the end of the experiment, the mean survival and mean RWG % in *H. tubulosa* (respectively 88.7 % and 13.5 %) resulted significantly higher than in *H. polii* (respectively 66.7 % and 0.62 %). When comparing growth performances among rearing densities, a clear trend was observed in *H. tubulosa* with significant differences among rearing conditions. In fact, the RWG % resulted lowest at High density, progressively rising with the decrease of rearing density (Fig.1). Instead, in *H. polii,* although the highest RWG % was also obtained at Low density, no significant differences emerge among rearing experimental conditions (Fig 1).

Discussion

The present study highlighted for the first time the different compatibility of *H. tubulosa* and *H. polii* in polyculture with fin-fish, evidencing that these two sea cucumber species have different feeding behaviors and culture requirements. *H. tubulosa* and *H. polii* adopt different trophic strategies in the wild feeding conditions. *H. tubulosa* actively select the organic matter from the surface sediment layer. Conversely, *H. polii* is able to dig into the sediment in order to feed on meiofauna, microorganisms, and organic detritus in the deeper sediment layer. However, this burrowing behavior may not be compatible with the physicochemical factors below the fin-fish farms (e.g. reduced oxygen availability, excessive organic sedimentation, excessive shell drop, nitrate release), which potentially affected the growth and survival of *H. polii* during this study. In conclusion, *H. tubulosa* resulted a better candidate than *H. polii* as extractive species in IMTA with fin-fish. In fact, at the end of the four-month experiment *H. tubulosa* was able to increase energy-use efficiency inside commercial farms, promoting productivity increase and rearing diversification. Furthermore, our results highlighted that the stocking rearing density significantly affected the growth performances of this sea cucumber species in IMTA system. Hence future investigations must consider the rearing density of sea cucumber species as a key aspect in aquaculture trials.

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Shewanella putrefaciens FISH PATHOGENIC STRAINS CONTAIN PLASMIDS THAT ARE ABSENT IN THE PROBIOTIC STRAIN S. PUTREFACIENS PDP11

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Introduction

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts. *Shewanella putrefaciens* Pdp11 has been described as a probiotic for farmed fish species such as *Solea senegalensis* and *Sparus aurata* Tapia-Paniagua et al., 2012). In contrast, other strains of *S. putrefaciens* have been described as pathogenic for other cultured fish (Esteve et al 2017)damage of the mouth, extensive skin discoloration, exophthalmia, ascites and bad odour. The S. putrefaciens group was recovered from freshwater samples taken at the L′Albufera system, along autumn–winter 2015. Its counts significantly increased in freshwater parallel to hypoxia and temperature rising. Shewanellae strains were identified as S. putrefaciens and S. xiamenensis by 16S rRNA gene sequencing. These isolates recovered from sick eels or freshwater were virulent for European eel by IP challenge (LD50 106 CFU g−1 body weight. The plasmids plays an important role in the genes transfer and insertion then there can been implicated in antibiotics resistance, degradative pathway and pathogenicity characteristics (Kornelia et al 2015). The genetic variation conducted by plasmid could induce an impact in probiotic proprieties. In this research, we searched the present or absent of plasmids in pathogenic and probiotic strains of *S. putrefaciens*. As well as, this plasmids implication in development of virulence factors.

Material and methods

Shewanella putrefaciens strain Pdp11 was isolated from skin mucosa of healthy gilthead seabream (*Sparus aurata* L.) (Chabrillón *et al.*, 2005) and *Shewanella putrefaciens* SH12, SH4, SH6, SH9, SH16 have been associated with diseases in eel (*Anguilla anguilla* L.)(Esteve, , 2017)damage of the mouth, extensive skin discoloration, exophthalmia, ascites and bad odour. The S. putrefaciens group was recovered from freshwater samples taken at the L′Albufera system, along autumn–winter 2015. Its counts significantly increased in freshwater parallel to hypoxia and temperature rising. Shewanellae strains were identified as S. putrefaciens and S. xiamenensis by 16S rRNA gene sequencing. These isolates recovered from sick eels or freshwater were virulent for European eel by IP challenge (LD50 106 CFU g−1 body weight.All *S. putrefaciens* strains were grown on 10ml of Tryptone soja broth supplemented with NaCl 1.5% (TSBs) for 24h at 23ºC. Given the possibility that, the hypothetical plasmids could be integrated into the bacterial genome, strains were cultured under different and strict growth conditions (temperature, incubation time, growth medium and freeze-thaw) to favor their excision. For this, all strains of the study were cultured in 10 mL tubes of TSBs and minimal media (M9), and incubated at 23ºC or 4ºC for 24h and 48 h on shaking at 80 rpm. Cultures inoculated in parallel with TSBs medium containing glycerol (20 %) were subjected to a freeze-thaw cycle for 24 h at -80 ºC before incubation. The positive control was *Escherichia coli* V157, a strain harbouring seven plasmids, grown on Luria Bertani broth (LB) for 24h at 37ºC under agitation at 80 rpm. The cultures were centrifuged and the pellet was used for plasmid DNA (pDNA) isolation. pDNA integrity was checked by agarose gel. Rolling circle amplification (RCA) was performed using the TempliPhi 100 amplification kit for each isolated plasmid following the manufacturer’s instructions.

The Illumina sequences were obtained by Seoane et al. 2019, and Plasmid-specific Sanger sequences from pDNA isolation and RCA amplification as above. Vector removing and quality trimming steps of Sanger reads were performed with BBDuk. Two sets of vector-free Sanger reads were obtained in the quality trimming. The low stringency threshold generates longer reads used to capture plasmid-specific Illumina reads and the high stringency threshold for the final assembly. Then, plasmids were assembled *de novo* using a workflow integrating Sanger and Illumina reads. Plasmid sequences were recircularized by the MARS method, and plasmids were aligned by the Clustal method of the Seaview program to finally obtain the plasmid consensus sequences. The plasmids annotation and functional characterization were performed. The sequence analysis showed that the plasmids encoded a putative replication initiator protein of the repB family, and proteins related to plasmid stability and a toxin-antitoxin system.

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Results and Discussion

Using a workflow integrating Sanger and Illumina reads, the complete consensus sequences of the plasmids were obtained. We found that the main ORFs conserved in both plasmids pSH4 and pSH12 belong to the replication protein initiator repB superfamily, and PemK/PemI family protein. The Rep proteins are especially important as they are primarily responsible for the initial DNA binding and nicking activities (Stolz, 2014). Another major ORF identified is the PemK protein as part of toxin-antitoxin (TA). This system is found both in bacterial chromosomes and in MEGs such as plasmids and prophages (Bukowski et al., 2019).

The presence of plasmids is expected to be associated with bacterial survival and, commonly, with virulence factors. The probiotic strain *S. putrefaciens* Pdp11 did not present plasmid, which was only found in two of the five pathogenic strains. The results allowed us to discard the probiotic Pdp11 could present a pathogenic characteristic as the TA type II system as a virulence factor and its self-regulating characteristics, which may be behind its probiotic nature, making the Pdp11 strain unique in comparison to other *S. putrefaciens* strains.

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BIOFILM INHIBITION OF PATHOGENIC STRAINS BY EXTRACELLULAR PRODUCTS (ECPS) OF *Shewanella .sp* PROBIOTIC

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Introduction

Shewanella putrefaciens Pdp11 and *V.proteolyticus* DCF 12.2 are strains isolated by our research group. *S. putrefaciens* Pdp11 has been described as a probiotic for farmed fish species such as *Solea senegalensis* and *Sparus aurata* [1].Although the probiotic potential is well described some studies reference to application of postbiotic as functional bioactive compounds produces by probiotics. Recent works have been focused in the identification of substances as alternative of anti-biofilm methods and their implication in surface attachment inhibition [2]. In this research, *S. putrefaciens* Pdp11 and *V.proteolyticus* DCF 12.2 have been cultured under different growth conditions (temperature, culture media and during 24 and 48 hours of incubation) and their extracellular products (ECPs) have been extracted and tested as potential postbiotics that affect the biofilm formation of several fish pathogenic strains.

Materials and Methods

The probiotic strains were grown on tryptic soja agar plates supplemented with NaCl (1,5%) for 24h at 23ºC. Then, one to two colonies were picked up in 10ml of tryptic soy broth supplemented with NaCl (1.5%) (TSBs) and incubated at 23ºC and 15 ºC for 36h (stationary phase). Then, extracellular products (ECPs) from solid medium3, 1 ml of the cultures were spread on TSAs plates as control condition. Another 1 mL was spread on plates containing: Pdp11 was culture in a medium with 25% of algae mix (diet 1) and supplement with a commercial diet (diet 2) were incubated to 23ºC for 24h. While *V. proteolyticus* DCF 12.2 was incubated in a commercial and algae mix diet (diet 3) and with 25% of algae mix (diet 4) to 15 and 23 º C during 48 and 24h respectively. After incubation, the ECPs were recovered with 5 ml of sterile phosphate buffer saline (PBS), and centrifuged (10000xg, 4ºC, 10 min) and the supernatant was filtered (0.22µm, pore diameter). ECPs were conserved at -80ºC until use. The biofilm inhibition was determined for the pathogenic strains: *S. putrefaciens* SH4, SH12, SH6 and SH9 4damage of the mouth, extensive skin discoloration, exophthalmia, ascites and bad odour. The S. putrefaciens group was recovered from freshwater samples taken at the L′Albufera system, along autumn–winter 2015. Its counts significantly increased in freshwater parallel to hypoxia and temperature rising. Shewanellae strains were identified as S. putrefaciens and S. xiamenensis by 16S rRNA gene sequencing. These isolates recovered from sick eels or freshwater were virulent for European eel by IP challenge (LD50 106 CFU g−1 body weight using 96 well plates by adding 90µl ECPs + 90 µl TSBs + 20 µl of pathogenic bacterial suspension adjusted to OD600nm ~ 0.5, per well. The plates were incubated at 23ºC during 24h. The biofilm formation assay was performed by crystal violet (CV) staining5"ISSN":"0044 8486","abstract":"The use of effective biocides as disinfectants is essential in aquaculture facilities. However, while most biocides act effectively on free-living planktonic pathogens, they are seldom useful against biofilms. In this study, we evaluate the biocidal efficacy and antimicrobial specific contact time of three disinfectants, Virkon™Aquatic (VirA and quantify at OD595nm in a plates reader. The results were analysis by two-way Anova method.

Results and Discussion

Two different ECPs concentration were assayed to check the potential inhibition of the biofilm formation, 0.45 and 0.25 µg protein/ml (Figure 1). The SH4 and SH12 pathogenic strains did not present biofilm formation in contrast to SH6 and SH9 strains. The biofilm of SH6 presented the highest inhibition at a concentration of 0,25 µg/ml of respect to control in each condition. On the contrary, the biofilm of SH9 presented inhibition by at 0,014 µg/ml of ECPs. Some authors describe that the biofilm inhibition can be mediated by soluble antimicrobial peptides (AMPs) secreted on ECP of probiotics strains [6]. This assay results evidence that Pdp11_Pmix_2324 ECPs have showed the most impact in the biofilm formation of pathogenic strains. Therefore, ECPs secreted by Pdp11 and *V.proteolyticus* DCF 12.2 are implicated in the inhibition to adhesion of pathogens on surfaces.

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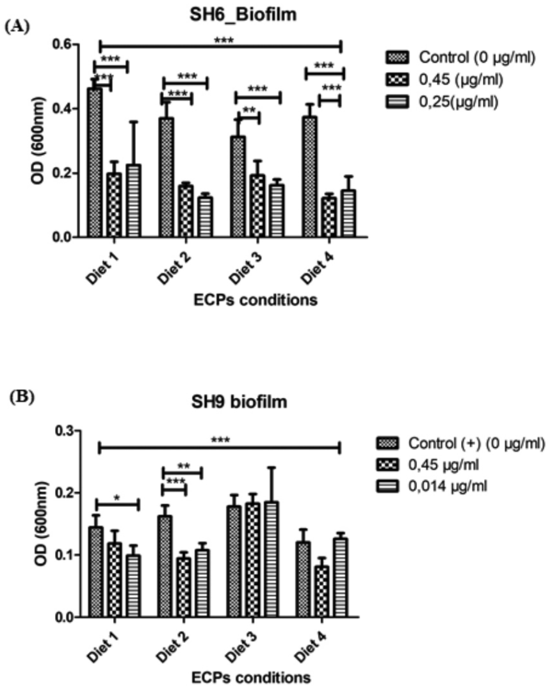

<u>Figure 1</u>. Biofilm inhibition in SH6 and SH9 pathogenic strains by *S.putrefaciens* Pdp11 and *V.proteolyticus* DCF 12.2 ECPs. The results were analysis by two-way Anova with P-value < 0.05 (*), P-value <0.01 (**), P-value < 0.001 (***).

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INITIAL RESULTS OF CHEMICAL INDUCTION OF METAMORPHOSIS IN THE PULLET CARPET SHELL CLAM, *Venerupis corrugata* (GMELIN, 1791)

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Introduction

Bivalve molluscs such as mussels, oysters, scallops, cockles, and clams are organisms of great ecological and commercial importance in Europe. The pullet carpet shell is a clam species very appreciated by consumers. Unfortunately, natural stocks decrease and the production of seed in hatcheries is hampered by different factors among which are broodstock conditioning, settlement and metamorphosis and diseases.

Clam larvae develop competence before recognizing appropriate exogenous cues to settle and metamorphose. Previous studies have shown the induction to metamorphosis using analogues of natural inducers in bivalve larvae from different species (Coon et al. 1985; Estupinan and Waite 1988; Dobretsov and Qian, 2003, García-Lavandeira et al. 2005; Alfaro et al. 2011; Mesías-Gansbiller et al. 2008, 2013). The aim of this study is to identify the chemical inducer capable of raising metamorphosis rates in pullet carpet shell clam larvae and in consequence to enhance their production and survival.

Materials and methods

The larvae were cultured in the facilities of the CIMA and the IGAFA. A semi-continuous larval culture system was used, with water renewal every 48 hours and feeding from axenic microalgae cultures. The water was treated with ultraviolet light. Three experiments of induction of the metamorphosis were carried out, with competent larvae of *V. corrugata* (between day 17 and day 20 of culture) using the protocols previously described in García-Lavandeira et al. (2005) and Mesías-Gansbiller et al. (2013). Competent larvae of *V. corrugata* were treated with several neuroactive compounds: GABA, L-DOPA, IBMX, serotonin, acetylcholine and the catecholamines epinephrine and norepinephrine. Three different concentrations of the potential inducers were used: 10^{-4}M, 10^{-5}M and 10^{-6}M with the aim of determining the optimal concentration. Experiments were carried out on a laboratory scale in Petri dishes of 90mm, at a density of 4 larvae/ml. Each experiment was performed in triplicate for each of the three concentrations mentioned above and also included a control with FSW (filtered sea water) and no potential inductor. After an exposure time to inducers or FSW of 72h larvae were observed with a Leica DM750 microscope. Larvae were considered to go through metamorphosis when they had a food to crawl and lost their velum. The percentage of metamorphosis was calculated by dividing the total number of larvae metamorphosed by the total number larvae and multiplying by 100. Percentages of both metamorphosis and mortality were analysed by ANOVA using the statistical package SPSS 20.0. The results were significantly different when $p<0.05$.

Results and discussion

Maximum percentages of settlement were observed with 10^{-4}M epinephrine, norepinephrine, and serotonin after 72 h of exposure to the inducer. These percentages were significantly different from the control with FSW. The percentage of larvae that passed metamorphosis in the control amounted approximately to 61%. Furthermore, exposure to 10^{-5}M epinephrine and serotonin also increase significantly metamorphosis. The percentage of metamorphosed larvae on *V. corrugata* obtained with 10^{-4}M acetylcholine was slightly significant comparing to the control larvae. In the cases of GABA and IBMX, they did not produce any positive or negative influence on the percentage of metamorphosis. By contrast, L-DOPA had negative effects on the metamorphosis rates, this compound resulted toxic for the larvae and reduce the percentage of metamorphosis comparing to the control larvae. Mortality of larvae was not affected by serotonin, epinephrine, norepinephrine and acetylcholine.

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As a consequence of these results, we can conclude that serotonin, epinephrine and norepinephrine are the best inducers of metamorphosis in *V. corrugata* larvae, although acetylcholine at high concentrations was also a good inducer. Acetylcholine and serotonin were also effective inducers of larval metamorphosis in the clam *Ruditapes philippinarum* larvae (Urrutia et al. 2004, own results). Furthermore, García-Lavandeira et al. (2005) found that epinephrine was an active inducer of metamorphosis in two clam species *Venerupis pullastra* (now named *V. corrugata*) and *R. philippinarum*, although the percentages of metamorphosis obtained by these authors were very low compared to those obtained in the study presented here. The different effect of chemical compounds in the induction of metamorphosis is clearly determined but not only by the species under study, as observed in the data obtained by different authors in different species of clams.

Acknowledgement

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BIVALVE AQUACULTURE UNDER VARIABLE ENVIRONMENTAL CONDITIONS AND ANTHROPOGENIC PRESSURE: A SURVEY OF TWO DISTINCT FARMING SITES IN THE EASTERN ADRIATIC, CROATIA

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Introduction

Bivalve aquaculture production in the eastern Adriatic is based on two species, namely, Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 and European flat oyster *Ostrea edulis* Linneaus, 1758. Sheltered bays and estuaries are preferred bivalve farming areas of optimal productivity in terms of nutrients and organic matter load. These sites display spatio-temporal heterogeneity of hydrological conditions and nutrients availability and are typically under risk from anthropogenic pollutants that may be delivered from land-based sources (Gavrilović et al, 2007; Milun et al, 2016; Šilović et al, 2012). As the overall performance of bivalves is tightly linked to marine ecosystem quality, it is necessary to evaluate whether these external stressors, alone or in combination, may affect the farmed organisms and consequently the seafood production success.

Materials and methods

This work was carried out in two distinct and geographically distant ecosystems of the Eastern Adriatic: Lim Bay and Mali Ston Bay, long been known for bivalve and fish farming. Both Lim Bay and Mali Ston Bay are legally protected marine reserve in the eastern Adriatic Sea. Samples of seawater, sediment and adult bivalves were collected bimonthly from June 2020 to May 2021 and processed in accordance to standardised protocols. Briefly, seawater parameters – temperature, salinity and oxygen saturation - were assessed immediately upon sampling of seawater. Total coliforms, *E. coli* and enterococcus as faecal indicators of microbiological quality were determined for seawater and sediment by substrate technology (IDEXX). Culturable heterotrophic bacteria in seawater, sediment and bivalve tissues were isolated on Marine agar and enumerated following 24-48 h incubation at 22°C. Chemical analysis of metals, PAHs, PCBs and pesticides in sediment and whole tissue was also carried out. Aliquots of seawater samples were stored and later used for total heterotrophic bacteria, chlorophyll a and particulate organic matter analyses. Condition index of mussels and oysters was determined according to Davenport and Chen (1987).

Results

Seawater physico-chemical parameters displayed typical seasonal variations, while microbiological quality and the content of chemicals displayed both seasonal and site-specific patterns. Faecal indicators in seawater and sediment, namely, total coliforms, *E.coli* and enterococci, occasionally displayed slightly higher values at Lim Bay site. A markedly higher levels of heterotrophic bacteria were recorded over almost the whole investigation period in samples of bivalves, seawater and sediment from Lim Bay. Concentration of metals and PAHs displayed species and site specific patterns, while the levels of PCBs and pesticides in both the sediment and tissues were below detection limits. Condition index of mussels and oysters also differed between two sites.

Discussion and conclusion

The overall results reflect specificities of each farming site arising from differences in nutrients enrichment and hydrological conditions at Lim Bay and Mali Ston Bay. Since further aggravation of environmental conditions driven by climate changes has been anticipated, the awareness of the policy makers and overall public on the necessity for preservation of coastal natural resources and vulnerable farming habitats will be crucial for sustainability and growth of eastern Adriatic bivalve aquaculture in the future.

Acknowledgements

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Chlorella vulgaris AND *Streptomyces rimosus* CO-CULTIVATION: DETERMINATION OF METABOLIC SHIFTS AND DESIRABLE BIOACTIVITIES

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Introduction

Co-culture systems are lately being explored as a potential replacement for monoculturing of various microorganisms in order to enhance the biomass yield, and increase synthesis of described and yet unidentified bioactive compounds. Utilization of microalgae-bacteria co-cultivation has been well documented in wastewater treatment and downstream processing such as biomass harvesting (Padri et al. 2022). In contrast, very little data is known considering the identification of novel metabolites produced only during co-cultivation. This area of research is of particular interest since most of these compounds have a great potential for medical and biotechnological applications. *Chlorella vulgaris* is a model microalgae intensively investigated due to its ability to produce various bioactive compounds beneficial for human health. Streptomycetes are well-known bacterial producers of antibiotics, anticancer and immunosuppressors. Abundantly present in the soil, they are adapted to various ecological niches. The capacity to produce diverse metabolites is a result of their interaction with various organisms. *Streptomyces rimosus* G7 is specifically known for its ability of oxytetracycline production.

Materials and methods

In this study, S.*rimosus* and C.*vulgaris* consortium was cultivated in Tryptic Soy Broth medium, under mixotrophic conditions. Regular monitoring of growth curves served to determine the most relevant bioprocess parameters under which these organisms co-existed. Extracted biomass, as well as metabolites excreted into the medium, were investigated in order to determine the significant effects on their metabolomic flux. Determination of biomass composition of both microorganisms has been performed using Nile Red (NR) method with fluorescence microscopy for lipid accumulation; spectrophotometry for pigments and the Lowry method for protein yield (Galić et al. 2021). Antimicrobial activity was tested using agar well diffusion method for detection of antibiotics (Petkovic et al. 2006).

Results

Co-culture systems resulted in an increased yield of neutral lipids which identification and quantification was furtherly studied using gas chromatography (GC). Interestingly, antimicrobial activity of S. *rimosus* as tested using the agar well diffusion method, and showed decreased antibiotic production in co-culture systems. Pigment content also decreased which can be linked to allelochemicals production, while protein composition remained the same.

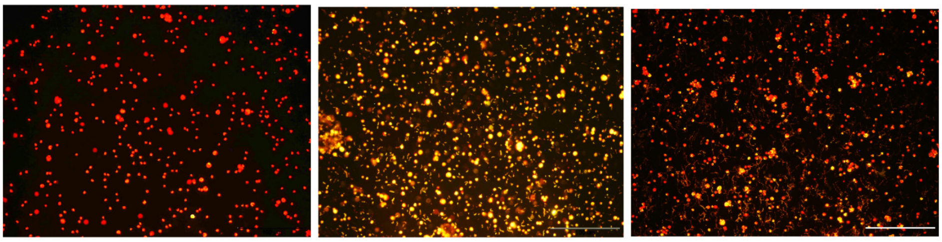

Figure 3. Fluorescence microscopy pictures of Nile Red stained lipids in *C. vulgaris* cells monoculture (a), *C. vulgaris* + *S. rimosus* (1:100 dilution) co-cultivation system (b) and *C. vulgaris* + *S. rimosus* (1:1000 dilution) co- cultivation system (c) on the last day of cultivation. Red stains represent polar lipids while yellow represent neutral lipid content.

Discussion

Optimization of bioprocess parameters of the co-culture system was successfully carried out in both Tryptic Soy Agar and Tryptic Soy Broth culture media. The total pigment content of chlorophyll a, b and pheophytin a, b was analysed regularly during co-cultivation. Moreover, the content of neutral lipids in the co-culture of *C. vulgaris* and *S. rimosus* (1:100 dilution) showed a significant increase in triolein production, which was 4,353 mg/mL on the last day of cultivation. These results indicate the potential of co-culture systems for biodiesel production and still need to be confirmed by gas chromatography (GC). Interestingly, the antimicrobial activity assay showed that oxytetracycline was not produced in both *C. vulgaris* + *S. rimosus* 1:100 and 1:1000 dilution systems. This specific metabolic shift in co-culture systems will also be further validated by high-performance liquid chromatography (HPLC). Metabolites synthesized and released in the culture medium, as well as those embedded in the biomass, will also be analysed by quadrupole time-of-flight (q-TOF) mass spectrometry to reveal the production of new compounds.

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THE EFFECT OF LIGHT INTESIVITY ON THE GROWTH AND SURVIVAL OF COMMON DENTEX *Dentex dentex* (L., 1758)

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Introduction

Common dentex (*D. dentex*) belongs to the family of the Sparidae. It is characterized by rapid growth, high meat quality and it is a very desirable candidate for aquaculture farming (Abellán, 2000). Common dentex rearing in the Republic of Croatia began in the 1990s but was not successfully implemented commercially. The assumption is that low survival rates were present due to inadequate food, breeding conditions and diseases (Efthimiou et al., 1994). One of the most frequent cause of common dentex's disease and mortality is stress sensitivity (Efthimiou et al., 1994). Due to lack of the data and insufficiently known facts about commercial common dentex breeding, but also the great breeding potential of this species and the economic viability of the product, we focused on studying the impact of abiotic factors that could be useful in the cultivation of this species (Abellán, 2000). The obtained results can expand the knowledge about the effects of abiotic environmental factors on the growth and survival of this species. Furthermore, this „*in situ*" experiment investigated how light intensity influence growth and survival of common dentex.

Materials and methods

The research was conducted in the middle Adriatic at Mala Lamjana fish farm, Bisage breeding site on the south side of the island of Ugljan, Croatia (Figure 1). D.dentex with initial average weight were distributed in four floating cages (9 x 5 x 5 m), 4 000 fish were placed in each cage.

Figure 1. Study area in the middle Adriatic at Mala Lamjana fish farm, Bisage breeding site on the south side of the island of Ugljan, Croatia.

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Experimental design included two groups of common dentex where the first group was shaded (K1 and K2) and the second group was exposed to natural light (K3 nad K4). Light intensity was measured with digital lux meter once per week, while temperature and oxygen were measured on a daily basis. Biometry samples were taken on a monthly basis and included total length, weight and condition index of the fish. In addition, mortality was controlled three times per week.

Results and Discussion

The experiment was conducted from January to June 2020. In the period of our research, sea temperatures oscillated from 13 ° C in February to 19 ° C in June when the sea temperature was the highest. Fish reared in cages K1, K2 were under the shade and cages K3, K4 were exposed to sunlight. The month of June showed the greatest improvement in the obtained average weight of all cages, K1 was the smallest, K2 and K3 were in a small difference from each other while K4 got the most on weight. The results we obtained do not confirm that light affects the growth of individuals because there is no statistically significant difference in the total growth of fish farmed under light and shade. Moreover, there is no negative impact of light founded on the fish growth and survival of common dentex exposed to light compared to the group of common dentex that were in the shade. However, this experimental results can be useful for better future selection of aquaculture technology for this new species.

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ASSESSMENT OF WELL-BEING OF FARMED EUROPEAN SEA BASS AND GILTHEAD SEA BREAM THROUGH A SMART OPERATIONAL TOOL

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Introduction

Animal welfare is a key issue for the sustainable and competitive development of food production systems, including aquaculture (Franks et al., 2021). Methodologies to score the welfare of farmed fish are under investigation to meet the raising interest for animal-friendly husbandry practices (Saraiva et al., 2019; Tschirren et al., 2021).

This work reports an application of the welfare scoring system developed in the H2020 PerformFISH project (Petochi et al., 2021), to assess the well-being of sea bass and sea bream farmed in EU using a dataset provided anonymously by aquaculture companies.

Materials and Methods

Welfare Assessment: on 191 batches accounting for 66 million of stocked fish (31 M sea bass and 35 M sea bream) farmed in 18 net pens farms; at the end of on-growing cycle (fish size 800g).

Operational Welfare Indicators (OWIs): animal-based and indirect OWIs, including mortalities (total, by diseases, at 3 and 10 days after first stocking), fish vaccination by diseases, antiparasitic and antibiotic treatments, feed intake, stocking density at harvest and discharged fish at slaughter.

Welfare Index: composite indicators approach (Adjusted Mazziotta–Pareto Index, AMPI), which consists in the aggregation of a certain number of elementary indicators representing different issues of the same multi-dimensional phenomenon (Mazziotta and Pareto, 2018).

Welfare Tool: PerformFISH Bass&Bream Welfare Scoring Tool (BBW-Tool)

Results

Welfare Index, as well as normalized OWIs values, fell correctly within the expected AMPI range of 70–130. Overall results showed a better welfare score in sea bass batches compare to sea bream (Fig. 1), with average W-Index of 101.3 (95% Confidence Interval: 100.1–102.5) and 92.7 (95% Confidence Interval: 91.6–93.8) respectively.

The multiple linear regression model highlights the significant effect of all the selected sea bass and sea bream OWIs on the W-Index (Figure 2) and their usefulness for assessing welfare during on-growing cycle. The percentage of vaccinated fish against *Photobacterium damselae* and *Vibrio anguillarum*, feed intake and mortality rate within the first 3 days after transport-stocking showed the higher effect on the W-Index. Vaccination is confirmed as the most influent OWI on the W-Index also when the analysis is performed separately for sea bass and sea bream.

Discussion and conclusion

The well-being of sea bass and sea bream can be measured at batch and farm level using the BBW-Tool, which represents a promising noninvasive methodology for benchmarking and improving standards of production (COM(2021) 236 final). Among composite multidimensional indicators, the AMPI resulted a good statistical tool for the holistic assessment of the well-being of farmed fish. The BBW-Tool is being implemented through smart solutions for data collection, analysis and visualization.

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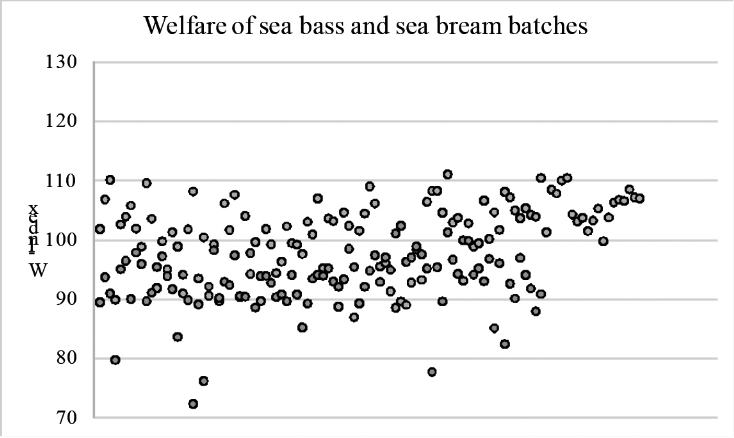

Figure 1. Welfare Index of sea bass (green dots) and sea bream (blue dots) batches farmed in net pens.

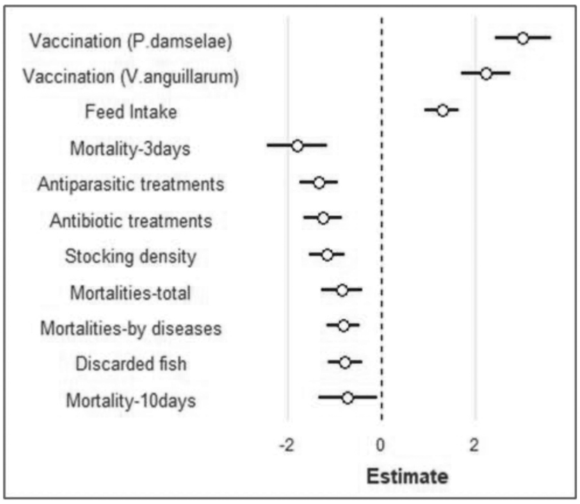

Figure 2. Effect of each OWI on the Welfare Index of sea bass and sea bream from the multiple linear regression model.

Acknowledgments

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A DIGITAL ERA FOR AQUACULTURE INNOVATION

Chiara Petrioli

University of Rome

Following a short video introduction of Big Data in the marine and maritime sectors, the plenary will then dive into applications developed by an Italian company WSENSE to provide innovative tools for blue marine sectors, with case studies from Northern and Southern Europe.

Chiara Petrioli - Full professor at University of Rome and founder of WSENSE
Chiara Petrioli holds a PhD in Computer Engineering from University of Rome La Sapienza where she is Professor of Computer Science and Engineering and directs two labs. She is a pioneer of the. Internet of Underwater Things.

Chiara is among the most cited international researchers in the field of computer science and engineering and has received several innovation awards -NT100 top social global techs changing our lives 2016; 100 women against the stereotype in STEM 2016; Inspiring Fifty Italy 2018; N2Women 2019 Stars in Computer Communications and Networking; Wired Italy fifty women who have made and are making the history of Computer Science; #EUWomen4Future, 100 women who are changing the world, Repubblica D 2021, Finalist Gamma Donna Award 2022.

“As a kid I was always fascinated by the abysses described by Jules Verne. Today I am exploring them with the technology I invented. Hold on to your dreams!”

Alexander Bergrem – WSENSE
Alexander Bergren is Vice President Sales & Business development, working for the Italian deep tech company, Wsense. Currently he is having the great task of commercializing wireless underwater communication technologies and underwater IoT systems supporting the Aquaculture industry towards even better sustainability and full digitalization.

He has 15 years experience from sales and 3+ years working with aquaculture tech companies in Norway, helping farmers bridge the technology gap (with great success.) WSense is one of top italian innovation companies (100 eccellenze italiane 2020) and winner of the European Commission Blue Invest Award 2022.

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Georgia Koutsandria - WSENSE
Georgia Koutsandria is Senior R&D Engineer at WSENSE where she is part of the team designing, implementing, optimizing and evaluating novel Internet of Underwater Things systems for different Blue Economy vertical sectors.

She holds a Ph.D. in Computer Science from “La Sapienza” University of Rome, an M.Sc. in Electrical and Computer Engineering from UC Davis, and a Diploma in Electronic and Computer Engineering from Technical University of Crete.

She has participated in European innovation projects in the Blue Economy field and to US projects funded by the US Department of Energy.

MONITORING OF BENTHIC INDICATORS OF ORGANIC ENRICHMENT UNDER AQUACULTURE SITES OVER MIXED BOTTOM SUBSTRATES DURING A FISH PRODUCTION CYCLE

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Norway's sheltered coastal and fjord systems provide an ideal environment for marine fin-fish farming, a growing industry worldwide. However, fin-fish aquaculture is associated with several environmental issues, including the deposition of organic waste (uneaten feed and faeces) to the seafloor and the consequent impact on benthic communities. In Norway, a system of monitoring investigations is already in use for the assessment of benthic organic loading and community responses for soft-sediment environments. However, not uncommonly on the Norwegian coast, grab sampling is made difficult by the presence of mixed- and hard- bottom substrates. The aim of this study was to detect the presence of benthic visual indicators of organic enrichment (organic pellets, sulphur oxidizing bacteria-mats, opportunistic polychaete complexes (OPC), the lugworm *Arenicola marina*, polychaete tube aggregations, epifauna) through image analysis, and to determine the environmental factors affecting their distribution. Changes in the epifaunal community structure through time, and over different locations and habitats (soft-, mixed-, hard- bottom substrate), were also investigated. Images were collected beneath 3 fish cages (and a reference site) at a fin-fish farm site on the Western coast of Norway. A method was developed to characterize images and provide quantitative information on the ecological state under the farm throughout a fish production cycle. Using the software Biigle 2.0, pictures were examined for surface area coverage of organic pellets and abundance of bacterial mats, OPC, *A. marina*, polychaete tube aggregations and epifauna. Results showed that the presence of organic material and bacterial mats was not indicative of a particular level of organic enrichment, as they were present throughout the whole fish production cycle. Benthic organisms such as the lugworm *A. marina* and aggregations of polychaete tubes were indicators of relatively low levels of organic enrichment and early stages of the production cycle. On the other hand, opportunistic polychaete complexes were indicators of relatively high levels of organic enrichment from the farm. Epifaunal community structure was considerably affected by the deposition of organic waste, as well by the sediment type, and taxa richness was considerably higher at reference sites (100 meters from farm cages). These results provide knowledge on the temporal impact of organic enrichment on benthic communities beneath fish farms over mixed- bottom substrates and highlight how the use of image characterization can improve the monitoring of benthic communities. The outcome of this study can contribute to the development of an environmental index to assess the ecological state around aquaculture farms placed over mixed- and hard- bottom areas.

ASTRAL POOL OF TECHNOLOGIES: DIGITAL TWINS TO SUPPORT MULTI TROPHIC AQUACULTURE WITHIN THE ATLANTIC AREA

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Introduction

Aquaculture is a fast-growing economic activity to boost sources of animal proteins worldwide, leading to significant industry investment[1]. The project All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) focuses on integrated multi-trophic aquaculture (IMTA) farming to support and promote sustainable aquaculture production across the Atlantic area. IMTA applications are exposed to many threats from external factors, such as climate change, increased risk of diseases[2], parasites and Harmful Algae Blooms (HABs)[3]. These activities also experience production and infrastructure risks from extreme weather events, changes in critical water physicochemical parameters (e.g. nutrients, temperature and dissolved oxygen). Besides, microplastics – an emerging pollutant – need to be carefully monitored because of impacts such as ingestion or filtration by aquatic fauna[4].

Supporting sustainable IMTA activities within the Atlantic area calls for a complete technological suite to best address end-user needs. The ASTRAL pool of technologies aims at supporting these activities through a set of innovative components, including an Artificial Intelligence (AI) data analytics platform, emerging sensors, cost-effective Internet of Things (IoT) kits, off-the-shelf cameras and hardware solutions. All technologies embed some form of intelligence with advanced deep neural networks trained and validated in relevant environment spanning across Brazil, South Africa, Scotland, Ireland and Argentina. The present abstract showcases such a pool of technologies and its associated benefits to IMTA applications.

Aquaculture Digital Twins

The ASTRAL project proposes developing a set of technologies to support IMTA applications. It aims to pilot emerging sensor technologies coupled with IoT/AI solutions to help and improve aquaculture activities while monitoring the surrounding environment. The proposed set of **Aquaculture Digital Twins** enables virtual replicas of the farming environment, species and facilities.

Cost-effective IoT Kits facilitate real-time aquaculture monitoring through multi-sensor twins for crucial water parameters (e.g., dissolved oxygen, temperature, chlorophyll-A, turbidity). The deployment at the aquaculture facilities extensively uses functional capabilities of long-range communication, optimised self-powering, improved software and sensor calibration models. Another type of monitoring twin leverages MEMS-based UV-VIS fluorometer-spectrometer to deliver complementary monitoring information ranging from nutrients to and physico-chemical parameters (e.g., ammonium, nitrate and dissolved oxygen).

AI Vision digital twins can benefit aquaculture farmers with automation to increase sampling capabilities and improve accuracy in visual-based inspection tasks. Work is underway to design and validate vision twins using cost-effective off-the-shelf cameras integrated into recent embedded AI GPUs for edge perception and processing in the aquaculture environment. The AI vision systems support macro-level animal biomass estimation on which AI models are trained specifically for multi-species fine-grained assessment of shrimps and urchins temporal profile growth. At a micro-level, the proposed AI vision twins provide a bench-top solution for HABs and microplastic monitoring, further customised with AI models trained for a diverse set of IMTA conditions (e.g. low to high water turbidity).

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Considering that the IMTA is exposed to several risks, a holistic water monitoring solution is key technology to ensure optimised farm operation. Biosensor technologies based on bivalve behaviour are sensitive solutions for many of the events of concern (e.g HABs, toxic compounds) and physicochemical monitoring parameters. ASTRAL technology employs bivalve behaviour data coupled with AI models as “proxies” for water quality assessment. The biosensing twin uses either vision or MEMS-based inertial sensors as the underlying technology for AI model training and real-time inference.

The AI data analytics platform (AIDAP) aims at integrating all aquaculture digital twins to operate in real-time supporting end-user decision making. The AIDAP is an analytics off-premise (e.g., cloud-based) architecture that hosts multi-modal time-series sensor data, AI models and visualisation dashboards for the end-users. The platform enables anticipation of scenarios and events using predictive modelling of physicochemical parameters and biological water quality indicators built upon the latest developments in data science. The platform connects all the aquaculture digital twins via web-based API interfaces. Sensor data is fed into this platform to develop and improve data modelling algorithms.

Conclusions

The ASTRAL pool of technologies provides a complete digital twin solution for IMTA applications. Technology validation is currently underway to improve the technology maturity level in the relevant environment (IMTA activities in Brazil, Scotland, Ireland, South Africa and Argentina).

Acknowledgements

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CLIMATE CHANGE AND AQUACULTURE

Nadia Pinardi

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An overview of the **short term scenarios** of how climate changes will affect temperature, sea level, severe weather, flooding, erosion, acidification for the Med and Adriatic. Followed by specific aquaculture climate impacts and challenges - with case studies of iconic fish and shellfish species.

Nadia PINARDI holds a Ph.D. in Applied Physics from Harvard University, and she is full professor of Oceanography at Bologna University. Her interests range from ocean numerical modelling and predictions to data assimilation, numerical modelling of the marine physical-biological interactions and pollutants at sea.

Her major achievement is the conceptual design and practical implementation of ocean forecasting systems across the world ocean: she started with the contribution to the very first real time ocean forecast in the California Current system to the complete development of monitoring, modelling and data assimilation components for the Mediterranean, Marmara and Black Sea.

From 2012 to 2019 she was co-president of the Joint Committee for Oceanography and Marine Meteorology (JCOMM) of UNESCO-IOC and WMO and she is, since 2019, elected vice-president of the Commission for Observation, Infrastructure and Information Systems (Infrastructure Commission) of WMO. She is chair of the UN Decade for Ocean Science Program “CoastPredict”. Nadia Pinardi was awarded in 2007 the European Geophysical Union (EGU) Fridtjof Nansen Medal for Oceanography, in June 2008 the Roger Revelle Unesco Medal, in 2015 she received the Italian Republic Honors “Commendatore Ordine al merito della Repubblica italiana” and in 2017 the Laurea Honoris Causa for Operational Oceanography at the University of Liege, Belgium.

Mariana Mata Lara - Geonardo Environmental Technologies
Mariana is a Senior Project Manager for Geonardo Environmental Technologies focusing on EU funded projects in the areas of Blue Economy and Science Education. She was the coordinator of AQUA-LIT project, focusing on developing with the aquaculture sector a toolbox to bring together existing, upcoming and implemented tools, case studies, best practices, policy recommendations and action plans for addressing the 3 main components of marine littering: prevention & reduction, monitoring & quantification, and removal & recycling. Currently, she is helping develop the Knowledge Exchange Platform for the COASTAL project, focused on bringing evidence-based solutions and policy recommendations for creating synergies and business opportunities between the coastal and rural areas in Europe.

PULSED ELECTRIC FIELDS (PEF) AND ACCELERATED SOLVENT EXTRACTION (ASE) FOR THE VALORIZATION OF SHRIMP BY-PRODUCTS: RECOVERY OF ASTAXANTHIN AND ANTIOXIDANT EXTRACTS

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Introduction

An important category of by-products from seafood processing includes crustacean ones. Approximately 6-8 million tons of crustacean waste is produced worldwide every year (FAO, 2014) Shrimp and prawns are one of the most important internationally traded seafood products, and one of the few that can be considered a "commodity", with a value of US$10 billion (or 16% of world fishery exports) (Gillett, 2008). Shrimp by-products represent important natural sources of carotenoid, among which astaxanthin (ASX) is the major one. Recently, pulsed electric field (PEF) treatment showed to be a promising method for the isolation and extraction of several components from seafood by-products such as calcium, chondroitin sulphate, collagen, chitosan, and protein (Bruno et al., 2019). Accelerated solvent extraction (ASE) is considered a green technique to recover bioactive and nutritional compounds in plants and food matrices (Sun et al., 2012). The main objective of the present study was to apply PEF and ASE using two different organic solvents (dimethyl sulfoxide, DMSO and ethanol, EtOH) to recovery ASX from shrimp by-products, and evaluate the effects of these technologies used independently or in combination on the ASX content and antioxidant activities of the extracts.

Materials and methods

In this study, fresh samples of red shrimp (*Aristeus antennatus*) and camarote shrimp (*Melicertus kerathurus*) were obtained from a local market in Valencia, Spain. Dimethyl sulfoxide (100% DMSO) or ethanol absolute (EtOH) were used as organic solvents. The combined and independent effects of the emerging technologies PEF and ASE using different solvents (EtOH and DMSO) on the extraction of astaxanthin were evaluated for each shrimp species. ASE (50 °C, 15 min, 103.4 bar) and PEF (3 kV/cm, 100 kJ/kg, 74 pulses) were used as extraction technologies. The antioxidant capacity of the extracts was evaluated by and Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays. Experimental data were subjected to one-way analysis of variance (ANOVA) to determine the significant differences among samples. Tukey HSD (Honestly Significant Difference) multiple range test, at a significance level of $p < 0.05$, was applied.

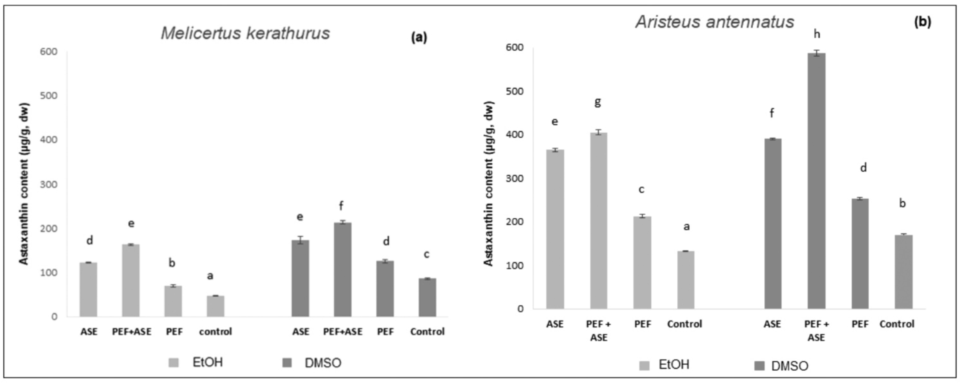

Figure 1: Astaxanthin content in *M. kerathurus* (a) and *A. antennatus* (b) by-products. Different letters above the bars indicate statistically significant differences between treatment averages ($P < 0.05$).

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Results

Preliminary results showed that ASE and PEF increased the astaxanthin content in the extracts for both shrimp species and solvent used, and the higher recovery was obtained using their combination (Fig 1).

Both technologies seem to be an effective tool to recover astaxanthin and antioxidant extracts from shrimp by-products. However these promising results should be confirmed by extending the study to other valuable compounds from crustacean by-products.

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INNOVATIVE CRYO-SMOKING PROCESS FOR THE PRODUCTION OF SMOKED SALMON

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Introduction

The demand for seafood has increased greatly due to their attractive nutritional properties but its rapid perishability remains the greatest challenge to its preservation. Therefore, an increasing number of emerging strategies are being considered to complement or even replace traditional preservation methods to ensure food safety and extend shelf-life (Tsironi, et al. 2020). Smoked salmon is an important added value 'ready-to-eat' food. It is estimated that about 40-50% of farmed Atlantic salmon reaches the final consumer as a cold-smoked product (Lakshmanan et al. 2003). The smoke contains several phenolic compounds that impart flavor to the product, the absorption of which depends on the temperature and smoking time (Arvanitoyannis and Kotsanopoulos 2012). The purpose of the present study was to evaluate an innovative cryogenic smoking technology to obtain ready-to-eat smoked salmon.

Materials and methods

Salmon (*Salmo salar*) used in the present study came from the Salmar farm (Kverva, Norway) where they were reared for approximately 12 months in floating cages in the open sea. After catching, fish were placed inside containers with polystyrene, stored in super-chilling and sent by plane to Italy. After 48 hours, they were filleted, vacuum-packed and frozen at -40°C and maintained at this temperature until salting and smoking procedures were performed. Thawed fillets were salted with a mixture consisting of 70% NaCl and 30% sucrose in a proportion equal to the weight of the fillet, vacuum-packed, and stored in cold storage at 4 ± 2°C for 24 hours. Dry-salted Atlantic salmon fillets were smoked using an innovative nitrogen-based prototype working at different temperatures (5, 20 and 45 °C) for 2 hours. Textural and colorimetric properties, NaCl content, water activity, water content, pH and polycyclic aromatic hydrocarbon (PAH) levels were determined. In addition, ^{1}H-NMR was used to monitor freshness-related parameters (e.g., K-index, trimethylamine content, biogenic amine content).

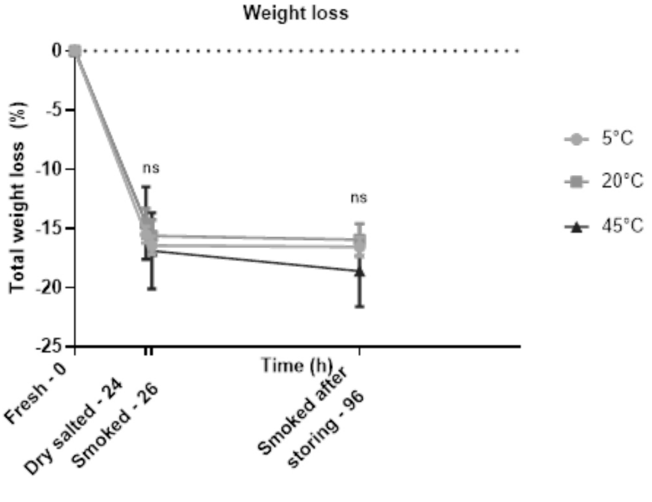

Fig. 1 - Kinetics of percentage weight loss of salmon fillets, fresh (0h), dry salted (after 24h), smoked at 5, 20 and 45°C for 2h and after 96h of vacuum-packed refrigerated storage.

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Results

For most of the parameters evaluated, the group of samples smoked at 45°C differed significantly from those obtained at 5 and 20°C. In particular, they were characterized by a greater weight and water loss, higher salt content, higher pH, lower shear force, increased lipid oxidation index, and a greater color change during the process with an increase in the red component. Between the samples smoked at 5 (cryo-smoking) and 20°C (cold-smoking), the only slight difference found was less weight loss of the samples during the process at the lower temperature (Figure 1).

An increase in TBARS values with the temperature rising has been evidenced, although the differences between the groups treated at 5 and 20°C were not significant.

The results allowed samples to be discriminated based on smoking temperature, and the innovative smoking process showed great potential for the preparation of high-quality smoked salmon product with high nutritional characteristics. The metabolomic approach provided a better understanding of the effect of smoking temperature on the quality of the final product. From a technological point of view, cryo-smoking is therefore promising for industrial application considering the lower weight loss of the product during the process. Further studies are needed concerning the shelf-life and microbiological quality of the final product. Moreover, it is advisable to investigate the organoleptic properties by sensory analysis in order to have a complete view of how the innovative treatment affects the quality of the finished product.

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NUTRITIONAL EFFECTS OF *T. verrucosa*-FED ROTIFERS IN THE GROWTH AND DEVELOPMENT OF ZEBRAFISH

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Introduction:

Zebrafish is a model animal widely used in many areas of scientific research. However, lack of standard feeding protocols has led to results difficult to reproduce when used in different facilities. Usually, in the laboratory, zebrafish are fed with a mixture of inert and live feed, such as brine shrimp or rotifers. As rotifers have a poor nutritional profile, their enrichment can be carried out with protein- and lipid-rich microalgae containing polyunsaturated fatty acids such as DHA or EPA. The values of these essential fatty acids can be further enhanced when microalgae are subjected to abiotic stress, such as temperature, salinity, and depletion of nitrogen and/or phosphorus.

This work aims to produce microalgae with an enhanced biochemical profile to enrich rotifers and meet the nutritional needs of zebrafish.

Methodology

In this study, *Tetraselmis verrucosa f. rubens* was cultured with Nutribloom Plus® in 1-L photobioreactors using two concentrations of nitrates, 2 and 10 mM. Rotifers were enriched daily with either *T. verrucosa* or Green Formula (GF, *Nannochloropsis,* Necton), the latter used as control. Zebrafish larvae were fed exclusively with rotifers enriched with microalgae or with control diet, Zebrafeed (Sparos). At 15 dpf, length, weight and condition factor were evaluated. At 30 dpf, survival, length, weight and condition factor, histology, skeletal deformations and biochemical profile of microalgae, rotifers and larvae were analyzed.

Results:

When analyzing the biochemical profile, there were significant differences in the lipid and protein content of the cells grown in the 2- or 10-mM nitrate growth media, with the latter being the best treatment for both parameters. Larvae fed exclusively with rotifers displayed a survival rate of over 90%, while larvae fed with inert food had a survival rate of only 56% (Figure 1). The incidence of skeletal deformities in the treatments of rotifers enriched with both GF and *T. verrucosa* grown in the 10 mM nitrate growth medium was 46%. Conversely, larvae fed with either *T. verrucosa* at 2 mM nitrate or ZF presented a higher incidence of skeletal deformities, 66% and 75%, respectively.

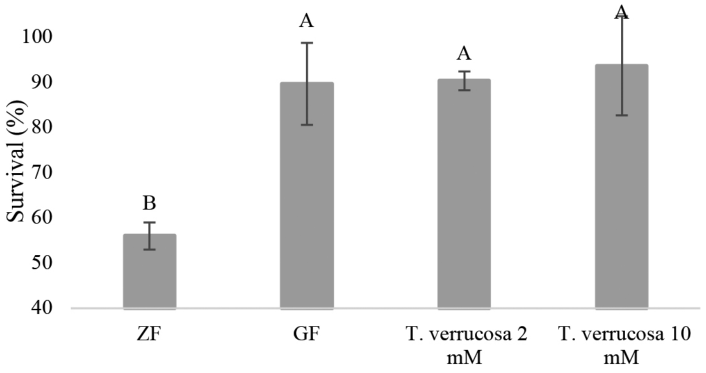

Figure 1 - Survival of zebrafish 30 dpf fed with inert food (**ZF**) and rotifers enriched with: Green Formula (**GF**), *T. verrucosa* cultivated with 2 mM nitrates (***T. verrucosa* 2 mM**), and *T. verrucosa* cultivated with 10 mM nitrates (***T. verrucosa* 10 mM**). Statistics: One-way ANOVA ($p \leq 0.05$). Different letters mean significant differences between samples.

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Discussion

The microalga *T. verrucosa* is a microalga rich in lipids (Cardoso et al, 2020), essential amino acids (Brown, 1991) and vitamins A, B and C (Fabregas and Herrero, 1990), all important for the development of zebrafish. In comparison, *Nannochloropsis* (GF) is a species rich in essential amino acids and polyunsaturated fatty acids, EPA in particular, which are essential for normal larval development (Liu et al, 2017; Chua and Schenk, 2017). This appropriate nutritional profile might have led to the observed decrease in skeletal deformation upon enrichment of rotifers fed with either GF or *T. verrucosa* at 10 mM, suggesting that high quality live feed is indeed beneficial for proper zebrafish growth and development (Kobayashi et al, 2008; Castaldi, 2019). Zebrafish have shown some growth delay when fed exclusively with microdiets, when compared to live feed. Moreover, inert feed has led to lower water quality by accumulation of debris, resulting in a significant decrease in survival (Harper and Lawrence, 2011).

In conclusion, GF-enriched rotifers are suitable for enhancing larval growth. Moreover, rotifers enriched with GF or *T. verrucosa* grown at 10 mM nitrates were able to decrease skeletal deformation in zebrafish larvae, while increasing dramatically larval survival.

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ON-DEMAND COUPLED FLOCPONICS SYSTEMS AS AN ALTERNATIVE APPROACH TO REDUCE THE PROTEIN LEVEL OF TILAPIA JUVENILES' DIET IN INTEGRATED AGRI-AQUACULTURE PRODUCTION

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Introduction

FLOCponics is an offshoot of conventional aquaponics in which the filter-based recirculating aquaculture system (RAS) is replaced by a system based on biofloc technology (BFT) (Pinho et al. 2022). Such replacement is usually justified by the potential benefits of BFT for tilapia juveniles culture and nutrition. Enabling the use of less protein in the fish diets is one of the great benefits of BFT (Emerenciano et al. 2021) as it favours the aquaculture sector in both economic and environmental terms (Bossier and Ekasari 2017, Emerenciano et al. 2021). The use of low dietary protein may result in (i) lower feed costs since protein is the most expensive nutrient in fish diets; (ii) lower use of fish meal and, on a large scale, minimizing the overexploitation of natural fish stocks; and (iii) decreased input of N and, depending on the production system, less discharge of N into the surrounding environment. This last consequence of reducing the amount of protein may also influence plant production in an integrated FLOCponics system. The effect of using less protein in the fish diet on plant growth must still be understood. Also, whether the nutritional benefit of BFT for tilapia juveniles production also occurs in FLOCponics has not yet been reported. Thus, this study was designed to investigate and evaluate the production of lettuce and tilapia juveniles in an on-demand coupled FLOCponics system using different levels of crude protein (CP) in the fish diets.

Material & methods

The zootechnical performance of tilapia juveniles and lettuce growth in on-demand coupled FLOCponics (FP) systems were evaluated, using different diets containing 24, 28, and 32% CP. Fish production in FP systems was compared to those reared in conventional on-demand coupled aquaponics systems and in stand-alone biofloc-based systems, both fed with 32% CP diet. The experimental period of tilapia juvenile production lasted 56 days. Lettuce production in two cycles was also performed in FP systems with different CP levels and their growth was compared to those in conventional aquaponics and hydroponics systems, as control treatments. In Cycle 1, the seedling phase was evaluated in a 14-day trial. In Cycle 2, the final production phase was performed for 21 days until harvest.

Results & Discussion

As expected, the results for tilapia growth demonstrated that the well-known benefits of BFT for juvenile nutrition are also found in the FLOCponics systems. The results showed that fish in FP-32 and BFT-32 grew similarly and 22.7% more than those in conventional aquaponics also fed with 32% CP. Additionally, tilapia reared in on-demand coupled FP and fed with 24 and 28% CP grew similarly to those in conventional aquaponics fed with 32% CP diet. These results suggest that even in an integrated system the *in situ* food present in biofloc-based systems is used by tilapia juveniles to complement their dietary protein needs. (Table 1). With respect to the question of whether DFP might enable lettuce production in comparable yields to conventional aquaponics and hydroponics, this study found no differences amongst the treatments for the growth parameters in the seedling (Cycle 1) and final production phase (Cycle 2). Interestingly, to achieve these similar yields, less commercial fertilizer was required in the DFP-32 compared to the other treatments.

Conclusion

The results obtained in this study indicate that using less crude protein in fish diets to produce lettuce and tilapia juveniles is technically possible and feasible in an on-demand coupled FLOCponics system.

(Continued on next page)

Table 1. Mean ± standard deviation of productive performance of tilapia juveniles during the 56-day experimental period.

Parameter	**FP - 24**	**FP - 28**	**FP - 32**	**BFT - 32**	**AP - 32**	***p*-value***
Zootechnical						
Final weight (g)	28.06 ± 2.04^{b}	29.63 ± 0.63^{b}	34.61 ± 1.66^{a}	34.76 ± 1.06^{a}	28.26 ± 0.50^{b}	< 0.001
Weight gain (g)	26.63 ± 2.05^{b}	28.2 ± 0.67^{b}	33.19 ± 1.60^{a}	33.37 ± 1.10^{a}	26.84 ± 0.57^{b}	< 0.001
SGR (% g day^{-1})	5.23 ± 0.13^{b}	5.33 ± 0.04^{b}	5.6 ± 0.08^{a}	5.61 ± 0.05^{a}	5.25 ± 0.03^{b}	< 0.001
Productivity (kg m^{-3})	4.88 ± 0.07^{b}	4.99 ± 0.18^{b}	5.86 ± 0.32^{a}	6.1 ± 0.28^{a}	4.71 ± 0.08^{b}	< 0.001
Total length (cm)	11.52 ± 0.34	11.66 ± 0.27	12.18 ± 0.25	12.1 ± 0.47	11.67 ± 0.13	0.098
Standard length (cm)	9.63 ± 0.29	9.7 ± 0.2	10.18 ± 0.25	10.16 ± 0.42	9.85 ± 0.24	0.127
FCR	1.07 ± 0.05	1.06 ± 0.11	1.02 ± 0.08	0.89 ± 0.03	1.05 ± 0.14	0.183
Survival (%)	96.59 ± 2.32	95.15 ± 0.57	90.31 ± 13.41	98.39 ± 0	95.4 ± 1.65	0.566
Protein-use efficiency						
PER	3.82 ± 0.19^{a}	3.3 ± 0.36^{ab}	2.98 ± 0.25^{b}	3.41 ± 0.11^{ab}	2.93 ± 0.41^{b}	0.021
PVV (%)	55.82 ± 2.93^{a}	50.71 ± 3.90^{ab}	48.32 ± 5.69^{ab}	58.43 ± 2.60^{a}	40.11 ± 5.70^{b}	0.004
CPwg (%)	14.65 ± 0.93^{ab}	15.43 ± 1.29^{ab}	16.17 ± 1.04^{ab}	17.14 ± 1.25^{a}	13.7 ± 0.24^{b}	0.017

* Means followed by different letters in the same line indicate statistical (one-way ANOVA at 5% significance level). FP: on-demand coupled FLOCponics system. BFT: bioflocs system. AP: on-demand coupled aquaponics system. SGR: specific growth rate. FCR: feed conversion ratio. PER: protein efficiency ratio. PPV: protein productive value. CPwg: crude protein on weight gain.

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BIOACTIVITY OF SULPHATED POLYSACCHARIDES EXTRACTED FROM SALT PAN BRINE WATER IN FISH

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Introduction

Sulphated polysaccharides (SP) are among the most abundant natural compounds produced by living organisms and are known for their antioxidant activity, immunomodulatory and anti-inflammatory properties[1]. They are highly soluble in water and can be found among Extracellular Polymeric Substances (EPS) present in high amounts in intertidal areas, where the marine organisms that excrete them can be found[2]. SP represent most of the organic components present in sea salt polymeric material[3] and Nunes *et al.*[4] have shown that salt pan brine water may be a suitable source of SP with immunostimulant properties that could have applications for fish health in aquaculture or research settings[4]. Zebrafish (*Danio rerio*) is an experimental animal model widely used in ecotoxicology but also to study processes related to fish farming[5,6]. In this context we studied the bioactivity of SP purified from brine water in zebrafish larvae, focusing on the effects over mineralization of the opercular bone. The immunogenic effect of SP was assessed using the Atlantic salmon (*Salmo salar*) head-kidney derived cell line SHK-1 through gene expression analysis.

Materials and Methods

Polymeric material was purified from salt pan brine water through four different methods, lyophilized and kindly provided by Nunes *et al.*[4] (University of Aveiro; Portugal). The amount of SP in each extracts – named SP1, SP2, SP3 and SP4 – were as follows: SP1, 311 mg/g; SP2, 293 mg/g; SP3, 313 mg/g; SP4, 324 mg/g.

Zebrafish were kept from egg fertilization to 3 days post-fertilization (dpf) larvae in 1-L breeding tanks (Tecniplast, Italy) with system water supplemented with 0.05% of methylene blue. Larvae were exposed in 6-well plates from 3 to 6 dpf to the different SP extracts at 0.0001, 0.0033 and 0.01% (w/v) and to negative controls made with artificial salt water (AS) and system water (NE – no exposure). At 6 dpf, larvae were stained with alizarin red S (0.025%) and imaged under a stereomicroscope Zeiss Axio Zoom V.16 equipped with a green fluorescence filter. Operculum area was determined through morphometric analysis using ImageJ macros[7,8].

SHK-1 cells[9] were cultured at 20ºC using Leibovitz's L-15 medium supplemented with 15% fetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine and 0.2% fungizone. Cells were seeded into a 6-well plate at a density of 5×10^5cells/well. After 48 h of incubation, cells were exposed for 4 h to 0.005% of SP extracts, 1 μg/mL of LPS from *E. coli* (positive control) or water (negative control), then RNA was prepared using NZYol. Expression of genes involved in the immune response (*tnfα, il6, il8*) and oxidative stress response (*cox2, sod*) were determined by qPCR from cell RNA using the ΔΔCt method.

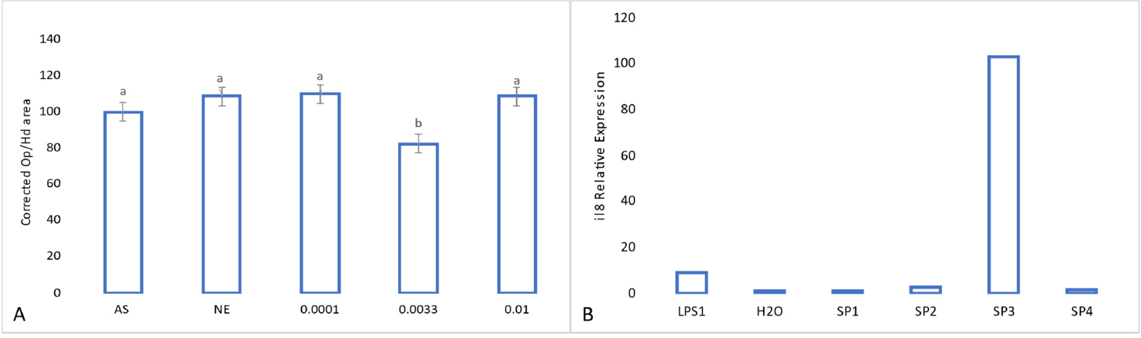

Figure 1. *A, Effects of SP3 treatment on corrected operculum area (Operculum/Head) in zebrafish larvae for different conditions: AS (n=45), NE (n=45), 0.0001 (n=43), 0.0033 (n=30), 0.01 (n=45). Different letters indicate statistical differences (one-way ANOVA, Tukey´s HSD, p < 0.05). B, Relative expression of il8 in SHK-1 cells exposed to SP3 (n=1).*

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Results

A preliminary toxicity test was performed with a broader range of concentrations on zebrafish larvae to establish the non-lethal concentrations. Operculum mineralization was not significantly altered in larvae exposed to SP extracts when compared to the negative control (AS). The highest values were observed for the SP3 treatment at 0.0001% and 0.01% (Figure 1A), while extract at 0.0033% resulted in a significant decrease in mineralization (possibly due to a smaller n). This suggests the existence of mineralogenic compounds in SP3. The exposure of SHK-1 cells to SP also identified SP3 as the most bioactive extract. It induced an overexpression of *tnfα, il6, il8 and cox2*, while expression of *sod* was not affected. This suggests a pro-inflammatory activity of SP3 extract, not observed in other extracts. The effects of SP3 on gene expression are particularly noticeable for the *il8* gene with a 100 times overexpression when compared to the negative control (H_2O; Figure 1B).

Discussion

SP3 extract slightly increased operculum mineralization, although not significantly, possibly indicating a pro-mineralogenic effect, as already reported for brown algae fucoidan (SP type) extract, which could accelerate the formation of calcium accumulation in murine mesenchymal cells[10], and triggers osteogenic differentiation in 7F2 murine pre-osteoblast cells and bone mineralization *in vivo* in mice[11]. Further studies should aim at testing higher concentrations and/or a longer exposure time in zebrafish. Our *in vitro* data indicate that SP3 may trigger a strong immunogenic effect, comparable to LPS effect. In that regard, we suggest that SP3 may have a similar effect to LPS that has been shown to stimulate salmonid lymphocytes proliferation, respiratory burst and phagocytic activity of macrophages in *in vivo* studies[12,13]. In addition, studies performed with SHK-1 cells showed an activation of innate immune markers upon LPS stimulation[14]. From the four fractions tested, SP3 exhibited the greater effect on gene expression levels showing pro-inflammatory effect. Further trials are being conducted with higher SP concentrations to understand if there is a dose related response. In addition, a concomitant exposure of LPS and SP molecules will be done to determine if SP could modulate LPS inflammatory effect.

Acknowledgements

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EFFECT OF A PROBIOTIC MIX ON FISH WELFARE AND STRESS RESPONSE: INFLUENCE OF THE TIME OF DAY OF ADMINISTRATION

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Introduction

In recent years, functional nutrition and nutritional biotechnologies have become fundamental to modern aquaculture practices. The inclusion of probiotics in fish diets has been shown to positively modulate the fish intestinal microbiota and immune status, reducing the incidence of pathologies and related medicinal treatments, and increasing somatic growth. Also, probiotics interventions can modulate the communication between the digestive and the nervous systems; i.e. microbiota-gut-brain axis (Cryan, 2011).

The aim of this study was to determine the effects of an in-feed probiotic mix of *Lactobacillus helveticus* and *Bifidobacterium longum*, with reported psychotropic-like properties, on behavioural and molecular stress biomarkers in a model fish species (zebrafish *Danio rerio*). In addition, given the existence of efficiency rhythms for drugs and medicines (Paschos et al., 2010), the influence of the time of administration was also studied.

Methodology

Wild-type adult zebrafish were divided into three experimental dietary treatments: Control (CTRL=standard diet provided both in the morning and in the evening); Probiotics-Morning (PB-M= standard diet supplemented with the probiotic mix in the morning and standard diet in the evening); Probiotics-Evening (PB-E=standard diet in the morning and the probiotics-supplemented diet in the evening) (n=30/dietary group). After 4 weeks, half of the fish from each dietary group were submitted to a stress challenge (air exposure for 1 min). Then, fish were allowed to recover for 30 min and both Stressed (S) and Non-Stressed (NS) groups were euthanized and brain samples collected to measure the expression of key genes involved in fish stress response and the GABAergic system. In addition, to evaluate the effect of the probiotic mix on zebrafish behavioural response, zebrafish from the same stock and subjected to the same dietary treatments (CTRL; PB-M; PB-E) were used to assess fish anxiety-like behaviours and locomotor activity with a novel tank-behavioural test, at two different times of the day (morning and evening; n=9/dietary group/time point), using Anymaze ® software. In both experiments, statistical differences were analyzed by two-way ANOVA (ANOVA II).

Results

In brain, a significant effect of the dietary treatment was found in the expression of genes coding for the glucocorticoid receptor (*gr*) and corticotropin releasing hormone binding protein (*crhbp*). For both genes, the expression levels were significantly lower in the PB-E group than in the CTRL, with the fish fed the probiotic mix in the morning (PB-M) showing intermediate levels (Fig. 1A and 1B). Regarding genes involved in the GABAergic system, a significant effect of diet was also observed for *gad2* (coding for the enzyme responsible for GABA synthesis) and *slc6a1a* (GABA transporter), which showed lower expression levels in the PB-M group in comparison to the CTRL. Besides, exposure to the stress challenge significantly increased the expression of *bdnf* (a neurotrophin which mediates behavioural responses in fish) compared to the Non-stressed group. As for the novel tank test results, significant effects of the dietary treatment were found on two anxiety-like behaviours analyzed: *Latency to top* and *Time in bottom.* Fish from both probiotic groups (PB-M and PB-E) took less time to enter for the first time at the top zone of the tank than the CTRL fish (Fig. 1C), whereas PB-M fish group spent less time at the bottom of the tank in comparison to the CTRL (Fig. 1D). In addition, some behavioural parameters were significantly affected by the time of the day in which the test was performed. Thus, *Mean speed* and *Travelled distance* were higher when the fish were exposed to the novel tank in the evening, whereas *Path efficiency* and *Latency to freezing* were lower in the evening than during the morning test.

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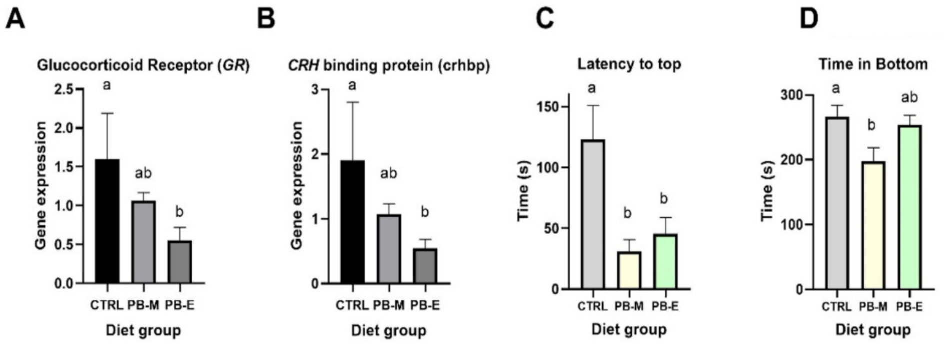

Figure 1. Effects of dietary treatments on zebrafish gene expression (A, B) and behaviour (C-D).

Discussion and conclusion

Feeding zebrafish with the probiotic-supplemented diet elicited significant effects at both behavioural and brain (gene expression) levels. Lower brain *gr* and *crhbp* expression observed in the PB-E group could indicate a lower activation of the HPI axis on these fish, suggesting that fish fed the diet supplemented with probiotics in the evening might present lower stress levels than those being fed only the standard diet. In addition, lower expression of both *gad2* and *slc6a1a* in the PB-M group might be related to lower levels of brain GABA, which would also reflect a lower activation of the HPI axis and lower stress levels in this probiotic-fed group. At behavioural level, the stress response triggered by transfer into the novel tank system was lower in the zebrafish fed the diet supplemented with probiotics in comparison to the CTRL fish irrespective of the time of administration (PB-M and PB-E groups), also pointing to an anxiolytic effect of the probiotic mix. In addition, exposure of fish to a novel environment was more stressful in the evening than in the morning, regardless of the dietary treatment. To conclude, these findings showed a positive effect of the probiotic mix on zebrafish stress response at both brain and behavioural levels opening the way to the potential application of psychobiotic to support animal health and welfare in the aquaculture industry.

Acknowledgements

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A FLOATING IN-SITU BIOREACTOR FOR THE PRODUCTION OF ALGAL BIOMASS THROUGH THE EXPLOITATION OF EXCESS NUTRIENTS IN AQUACULTURE UNITS

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The mankind stands on the edge of a technological revolution that will fundamentally alter the way we live, interact, and consume. It is not clear yet how it will unfold, however, one thing is clear: it must tackle the challenge of a rapidly increasing global population and its subsequent increased nutritional needs. The nutritional resources of the planet are reaching to a limit and already the 9.9% of the population is facing food insecurity and all forms of malnutrition, based on the 2021 report by the Food and Agriculture Organization of the UN. It is not only the low "quantity" of the available food, but it is also the low "quality", the deficiency of macro- and micronutrients, known as Protein-Energy Undernutrition (PEU).

Fish provides about 16% of the total animal protein consumed by humans globally and, in addition, it is an important source of essential minerals and fatty acids. Therefore fisheries and aquaculture industries are playing a central role in meeting food and nutrition security goals (Béné et al., 2015). However, the production cost, to make fish affordable for low-income people, and the maintaining, or even restoring, of the environmental sustainability are the main challenges of the sea-food-related industries that have to be meet (Béné, Barange, Subasinghe, Pinstrup-Andersen, Merino, Hemre and Williams, 2015).

The production of microalgal biomass, including eukaryotic organisms and photosynthetic cyanobacteria, could tackle the global PEU as it provides high-value biomass, while the existing diversity of microalgae species allows the production of different types of products. The advantages of using marine and freshwater microalgae species as nutritional supplements in human diet include: (1) the much higher concentration of proteins, compared to animal and plant biomass, (2) the presents of all valuable amino acids, (3) high concentration of lipids and fatty acids, (4) increased collection of vitamins and (5) antioxidants (Chen et al., 2019, Fields et al., 2020, Torres-Tiji et al., 2020). Clearly, several algal species are nutritionally complete foods, and in addition, their yields outperform most plant crops, and also are less vulnerable to climate change effects compared to animal and plant production. Moreover, cultivating microalge could allow the remediation of wastewaters from various urban and industrial sites, in addition to biomass production (Abinandan et al., 2018).

Currently, the available systems for the production of algal biomass are almost exclusively land-based, consuming valuable land, water and other resources. In addition, the cost of biomass production is still high compared to the market value of most production compounds (Araújo et al., 2021, Vázquez-Romero et al., 2022). Therefore, a cheaper with low environmental footprint algal cultivation method is needed.

In the frame of the EU & Greek-funded research project "Innovative Development of Multitrophic Aquaculture", funded by the "Innovation in Fisheries" EU-Greece Operational Program of Fisheries and Maritime, EPAL 2014–2020 (grant number 5029294), we designed and constructed a novel in-situ floating bioreactor (isFlBR) for the cultivation of marine microalgae.

The main construction material of the isFlBR is plexiglass® due to its increased transparency and mechanical strength for in-situ applications. The edges of the plexiglass® tubes are covered by thin albeit high-strength PVC rings which host the planktonic nets and filters that are needed to keep the micro-algal cells inside the tubes. The arrays of isFlBR tubes are placed in close proximity to the aquaculture cages in order to exploit the maximum of the dissolved nutrients that will are released to the water column.

The ongoing research activities include the cultivation of 3 micro-algal species with increased nutritional and biotechnological value *(Chlorella sp, Isochrysis sp, Nannochloropsis sp*) in both meso- and oligotrophic marine environments next to established aquaculture production units in Central and Southern Greece.

It is clear that the algal cultivation inside the natural marine environment cannot compete the high-yield biomass and constant production of the land-based systems, in terms of total biomass, since no extra nutrients, CO2, radiation is provided. In addition the environmental conditions are very variable over seasons not allowing constant and predictable production rates. Moreover, the in-situ cultivation of microbes makes it more vulnerable to contamination. However, (1) the low installation and running costs of such system, (2) the low environmental impact production due to no usage of land, water or other limiting resources, (3) the remediation of the water column and the production of oxygen during photosynthesis which could decrease the environmental footpring and increase the fish production of the aquaculture, (4) the potential of cultivation of algal species and/or biotechnological interesting metabolites that are not possible in-vitro; makes the use of inFlBR as an excellent supplementary algal production method which could provide additional financial and environmental benefits to the aquaculture industry.

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COMPARISON OF INTEGRATED PRODUCTION OF TILAPIA, SEAWEED AND WHITE SHRIMP IN BIOFLOC SYSTEMS DOMINATED BY CHEMOAUTOTROPHIC AND HETEROTROPHIC BACTERIA

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Introduction

The integrated multitrophic system emerges as a more sustainable alternative to traditional biofloc systems. The purpose of integrating shrimp and fish is to ensure that the fish can consume excess of bioflocs and use the microbial protein for their growth. The present study aimed to evaluate the performance of *Litopenaeus vannamei* shrimp and Nile tilapia *Oreochromis niloticus* in an integrated multitrophic culture with a biofloc system approaching the heterotrophic and chemoautotrophic pathways.

Methodology

The experiment was carried out at the Marine Aquaculture Center, Institute of Oceanography, Federal University of Rio Grande – FURG/ Brazil, for 75 days. Each system consisted of a 17 m^3 tank where the shrimp were allocated (400 shrimp/ m^2), a 4 m^3 tank where the tilapia were allocated (45 fish/m^3) and 1 m^3 were introduced 3 kg of *Ulva lactuca*. These tanks were interconnected and the water circulated between them by submerged pump and the water returned by gravity. There are two treatments: Chemoautotrophic and Heterotrophic, with three replications each. Shrimp had an initial weight of approximately 1g and tilapia 25g.

In the chemoautotrophic treatment was fertilized with inorganic compounds, the system water was matured to stimulate the development of nitrifying bacteria (Ferreira et al., 2021). In the heterotrophic treatment, ammonia was controlled by fertilization with organic carbon (sugarcane molasses) after storage of the animals, when the concentration reached 1.0 mg/L or. The animals were fed twice a day (8:00 am and 4:00 pm), the tilapia were underfed with 1% of the tank biomass to stimulate the consumption of bioflocs (Poli et al., 2019).

Results

The mean values of temperature, dissolved oxygen, pH, alkalinity, total suspended solids (TSS) and settling solids (SS) were equal between treatments. The mean values of nitrite (Figure 2) and ammonia were higher in the heterotrophic treatment. The mean values of nitrate and phosphate were higher in the chemoautotrophic treatment, as well as the clarification time (Table 1). The zootechnical performance of shrimp was better in the chemoautotrophic treatment. Only FCR and survival showed no significant difference between treatments. There was no significant difference in any of the zootechnical parameters for the fish.

The results regarding algae are being processed and could not be incorporated until now.

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Table 1. Water quality (mean and sd) results in the tratments chemoautotrophic and heterotrophic.

	CHEMOAUTOTROPHIC	HETEROTROPHIC
Ammonia (mg/L)	0.74±1.27^{b}	1.44±2.38^{a}
Nitrite (mg /L)	3.07±3.72^{b}	8.01±8.36^{a}
Nitrate (mg/L)	78.88±4.83^{a}	47.18±3.89^{b}
Phosphate (mg/L)	3.44±0.31^{a}	2.50±0.18^{b}
Alkalinity (mg/L CaCO3)	178.79±14.9	161.86±2.51
TSS (mg/L)	285.72±93.07	313.10±103.88
SS (ml /L)	10.06±7.56	10.37±6.78
Clarification time (hours)	160^{b}	240^{a}

TSS: Total solid suspended. SS: Settleable Solids. Different letters indicate significant difference between the treatments by t-t.

Table 2. Shrimp and fish performance after 75 days.

	Chemoautotrophic	Heterotrophic	Chemoautotrophic	Heterotrophic
	Shrimp		Fish	
Final mean weight (g)	11.24 ± 1.97^{a}	8.25 ± 1.50^{b}	171.62 ± 17.81	180.28 ± 9.23
FCR	1.15 ± 0.06	1.23 ± 0.15	1.13 ± 0.61^{a}	0.75 ± 0.14^{b}
SGR (g.week $^{-1}$)	0.85 ± 0.03^{a}	0.60 ± 0.03^{b}	12.22 ± 1.48	12.98 ± 6.77
Biomass (kg)	60.51 ± 2.89^{a}	40.41 ± 4.68^{b}	18.22 ± 8.00	22.21 ± 3.88
Yield (kg.m^{-3})	5.06 ± 0.12^{a}	3.65 ± 0.12^{b}	1.11 ± 0.37	1.29 ± 0.19
Survival (%)	95.5±5.8	94.3±2.2	75.05 ± 24.42	83.81 ± 8.93

FCR: Feed conversion rate. SGR: specific growth rate. Different letters indicate significant difference between the treatments by t-test.

Discussion

For maturation of the chemoautotrophic system, inorganic fertilizations with ammonium chloride and sodium nitrite were performed for 52 days, with the objective of maturing the nitrite-oxidizing bacteria in the system, before the animals were stored. The objective of the predominantly chemoautotrophic system is for the ammonia excreted by the animals to be converted into nitrate through nitrification. When compared to the predominantly heterotrophic system, which converts ammonia into microbial biomass, this process generates about 40x less total suspended solids (Ebeling et al., 2006). The previous inorganic fertilization in the chemoautotrophic treatment was efficient to keep the levels of nitrite low and this reflected in the better zootechnical performance of the shrimps. With higher biomass, consequently, the feed supply was higher in the system, reflecting higher TSS concentrations. It was not possible to identify a significant difference in the consumption of TSS by the fish, but the FCR lower than 1 in the heterotrophic treatment, without statistically differing from the chemoautotrophic treatment, indicates that the animals used the bioflocs as a food supplement. The chemoautotrophic system showed better shrimp productivity, but the tilapia performance was better in the heterotrophic treatment.

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BIOLOGICAL RESPONSES OF OYSTER *Crassostrea gasar* WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF TOTAL SUSPENDED SOLIDS IN A BIOFLOC SYSTEM: BEHAVIORAL AND BIOCHEMICAL ANALYSIS

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Introduction

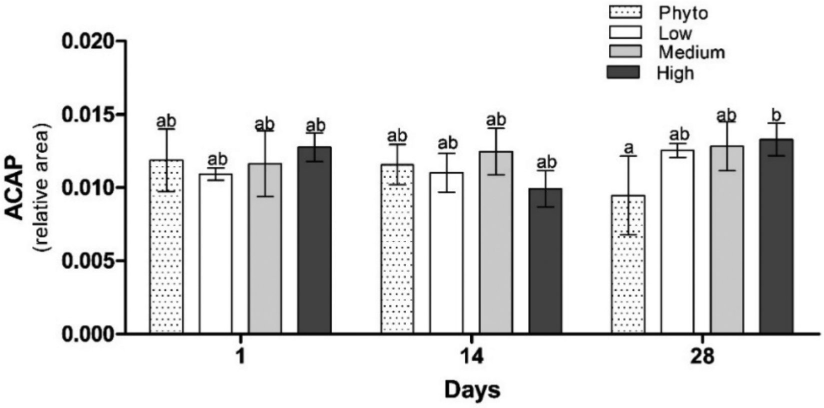
Oysters are known filter feeders and can play a role as consumers of bioflocs in integrated farming with other species (IMTA system). However, the tolerance of these bivalves to the high levels of total suspended solids (TSS) characteristic of cultures in biofloc systems is not known. Thus, the aim of the present study was to evaluate the biological responses of the oyster Crassostrea gasar when exposed to different concentrations of total suspended solids in biofloc culture systems, based on behavioral and biochemical analyses.

Methodology

Oysters with an initial weight of 54.18 ± 13.45g; height of 68.2 ± 4.44 mm; length of 50.4 ± 3.50 mm and width of 24.1 ± 5.02 mm and were cultivated for 28 days in 100L tanks. Four treatments were studied. Phyto: control treatment supplied daily with the microalgae Chaetoceros muelleri at a concentration of 16 x 104 cel/ml; Low BFT treatment – with maintenance of approximately 100 mg/l of TSS; Medium BFT treatment – maintained above 100 to 200 mg/l TSS; High BFT treatment – maintained above 200 mg/l TSS, each with 4 replicates.

The oyster valve activity was monitored using Hall effect sensors according to the methodology of Guterres et al. (2020). The total antioxidant capacity against peroxyl radicals (ACAP) was determined according to the methodology of (Amado et al., 2009).

Figure 6: Antioxidant capacity (relative area) in the gills of C. gasar oysters in the different treatments over a four-week experiment. The bars represent the mean values and vertical lines at the top of the bars represent the standard deviation of the values. Lower case letters in different days and treatments represent significant differences ($p<0.05$).



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Results

The use of aperture sensors indicated that there was a significant difference between treatments. The BFT High treatment had a shorter valve opening time, followed by the BFT Medium, BFT Low and control (Fito) treatments (Table 1). Table 1 shows the frequency (%) with which oysters open their valves and were classified as "Closed", "Slightly Open", Open and "Fully Open" (Table 1).

Regarding the total antioxidant capacity against peroxyl radicals (ACAP), there was a significant difference between treatments at the end of the experiment. The BFT High treatment showed a larger average area than the Fito, while the BFT Low and BFT Medium treatments showed no differences between the other treatments (Figure 1).

Discussion

The results demonstrate that oysters remain closed longer when the concentrations of total suspended solids are high. Although the high concentrations are within the ideal range for shrimp culture in a biofloc system (Samocha and Prangnell, 2019), for oyster insertion in an integrated system with shrimp, for example, these values should be kept at up to 200 mg/L. Stressful conditions in culture tanks can cause physiological changes including metabolic changes, decreased antioxidant capacity and oxidative stress, with consequences that can lead to the death of organisms (Romano and Zeng, 2013). After 28 days of culture, a reduction in the total antioxidant capacity (ACAP) of oyster gills in the BFT Alto treatment was also recorded, showing a break in the normality pattern compared to the control. Possibly, the valve closing behavior in the High BFT treatment resulted in the reduction of the total antioxidant capacity, when at week 3 there were limitations in the opening of the oyster valves, and influencing the biochemical responses of the bivalves.

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EVALUATION OF THE GROWTH OF MACROALGAE *Ulva lactuca* AT DIFFERENT DEPTH IN THE INTEGRATED MULTITROPHIC CULTIVATION WITH SHRIMP AND FISH IN BIOFLOCS

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Introduction

Cultivation in bioflocs (Biofloc Technology – BFT) is formed by aggregates of bacteria and other microorganisms that carry out the transformation of nitrogenous compounds in the system, with the accumulation of nitrate, phosphate and total suspended solids during the course of cultivation (Krummenauer et al, 2011). The presence of these nutrients in the system favors the insertion of macroalgae in integrated cultivation, which may lead to an increase in macroalgae biomass, nutrient absorption and an increase in the system's productivity. However, due to the high load of organic matter in the system, little is known about the influence of luminosity in the water column for the cultivation of macroalgae. The main objective of the study is to evaluate the effect of different depths of structures on the growth of the macroalgae *Ulva lactuca* in an integrated system with *Litopenaeus vannamei* and *Oreochromis niloticus* in bioflocs.

Materials and methods

The experiment was carried out in an agricultural greenhouse in 70 days of cultivation, consisting of six systems, each system was formed by a tank of 15 m^3 of useful volume with shrimp, and two tanks with 3 m^3 of useful volume with fish and macroalgae, separately. The density used was 300 shrimp/m^3, 35 fish/m^3 and 0.1g of macroalgae/L. Two treatments were performed with three replicates, namely: IMTA R shallow float (up to 10cm) and IMTA F deep float (up to 25cm), made of PVC pipes and 5mm mesh polyethylene nets. The initial average weight of the algae was 2.40±1.21 kg, weighing every two weeks to perform the Specific Growth Rate (SGR).

Water quality monitoring was performed, with temperature, dissolved oxygen and pH measured daily, and salinity and alkalinity were measured once a week. Nutrients and solids were performed twice a week, namely, total ammoniacal nitrogen, nitrite, nitrate, phosphate, total suspended solids (TSS), turbidity and settling solids (SS).

Data normality and homoscedasticity were verified using the Shapiro-Wilk and Levene tests, respectively. Once the assumptions were met, a t test was performed to verify the difference between treatments. A minimum significance level of 5% ($p<0.05$) was applied in all analyses.

Table 1 – Water quality parameters and macroalgae performance in integrated culture with shrimp and fish, in 70 days of cultivation.

Parameters	Shallow	Bottom
Seaweed		
Initial mean weight (g)	2401,00±0,20	2401,00±0,10
Final mean weight (g)	2629,33±0,227^{a}	1942,00±0,201^{b}
RGR (%/dia)	0,14±0,14^{a}	-0,35±0,17^{b}
Qualidade de água		
TAN (mg/L)	0,18±0,20	0,15±0,19
Nitrite (mg/L)	2,16±2,38	1,29±1,30
Nitrate (mg/L)	64,40±28,39	61,71±24,17
Phosphate (mg/L)	5,99±4,24	5,43±3,78
Turbidity (NTU)	219,49±86,66	196,02±68,72
SS (ml/L)	3,88±2,09	5,01±2,27
TSS (mg/L)	307,92±87,48	303,43±91,60

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Results

As a result, the first and second IMTA R weighings showed a TCE of 0.95±0.54 and 0.23±0.78%/day, respectively, with a significant increase in biomass. However, IMTA F treatment showed biomass loss -1.06±0.43 and -0.39±0.48%/day also in the first and second. On the other hand, in the penultimate weighing, TCE was negative for both treatments, with loss of biomass, with no statistical difference between treatments (-0.58±0.19 and -0.63±0.84).

There was no significant difference in water quality between treatments, with temperature, dissolved oxygen, pH, salinity and mean alkalinity of 22.29±0.01, 6.71±0.01, 8.03±0.04, 19 .46±0.65 and 219.02±1.24.

Discussion

Due to the high turbidity and solids in the biofloc system, there is little light penetration into the water, therefore, a shallower depth of structure increases the light absorption capacity of macroalgae due to their proximity to the surface. A decrease in temperature to 15°C was noted in the fourth week, which could be a stress factor for the macroalgae and thus initiate a reproductive event, as an indication of "ghost tissues" were found in the macroalgae tissue, as found by Da Silva et al. there, (2009).

With the exception of temperature, which remained low for shrimp farming (Wyban et al, 1995), the parameters were in optimal conditions for the cultivation of the species. The absorption of nutrients by macroalgae was not significant due to the low density of macroalgae used in the system.

Conclusion

Therefore, we can conclude that to improve the growth of macroalgae in integrated culture with shrimp and fish in a biofloc system, the use of shallow floats up to 10 cm are more efficient.

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DIFFERENT INCLUSIONS OF MACROALGAE *Ulva lactuca* IN THE DIET OF NILE TILAPIA

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Introduction

The use of alternative ingredients is a relevant area in the nutrition of aquatic organisms. In this sense, the macroalgae *Ulva lactuca* is an excellent candidate due to its antioxidant concentration, fatty acid and amino acid profile (Shuuluka et al., 2013). Water obtained from different environments can provide changes in the biochemical and nutritional composition of macroalgae (Duke et al., 1989). Therefore, the cultivation of macroalgae in biofloc systems with high concentration of nutrients and solids can change the nutritional composition of macroalgae. The aim of this study was to evaluate different inclusions of *Ulva lactuca* macroalgae produced in Biofloc system in the Nile tilapia diet *Oreochromis niloticus*.

Materials and methods

The experimental design consists in the use of three levels of macroalgae inclusion: 5, 10 and 15% *U. lactuca* in diet and a control treatment, without macroalgae, all with three replicates (Table 1). The experiment lasted 35 days and was performed at Estação Marinha de Aquacultura – FURG, with controlled temperature, at salinity 15. Ten juveniles with initial average weight of 2.12 ± 0.02g were stocked per experimental unit (50 L useful volume). The feed frequency used was 3 times/day, with 6% of the total biomass. Feces and uneaten food were siphoned off every day, and water changes were performed twice a week. Temperature, dissolved oxygen, pH, TAN, and nitrite were measured every day. Weighing measurements were performed weekly to adjust the amount of feed and to determine the feed conversion (FCR) and specific growth rate (SGR). The normality and homoscedasticity of the data were verified by the Shapiro-Wilk and Levene tests, respectively. All the data were performed with a one way ANOVA.

Table 1: Ingredient composition of experimental diet (% dry matter) containing *Ulva* meal in different levels.

Ingredients	**Experimental diets**			
	Control	**5%**	**10%**	**15%**
Fish meal	40.00	40.00	40.00	40.00
Soybean meal	33.00	32.00	31.00	30.00
Wheat bran	12.00	8.00	4.00	0.00
Gelatin	2.00	2.00	2.00	2.00
Soy oil	2.00	2.00	2.00	2.00
Fish oil	2.00	2.00	2.00	2.00
Cellulose	4.00	4.00	4.00	4.00
Vitamin/ mineral mix	5.00	5.00	5.00	5.00
Ulva meal	0.00	5.00	10.00	15.00
Proximal composition (%)				
Crude protein	42.44	41.82	41.37	39.98
Crude lipid	8.23	8.07	8.05	8.64
Ash	16.28	17.45	19.01	20.02
Gross energy (MJ/kg)	17.63	17.34	17.32	17.42

Table 2. Growth parameters and survival rate of Nile tilapia fed different levels of *Ulva* meal.

	Treatments			
EXPERIMENT	**Control**	**5%**	**10%**	**15%**
Average weight final (g)	9.21 ± 0.31	9.60 ± 1.35	8.78 ± 0.48	8.28 ± 1.84
SGR (%/day)	5.22 ± 0.15	5.35 ± 0.45	5.07 ± 0.19	4.84 ± 0.47
FCR	1.19 ± 0.09	1.24 ± 0.11	1.62 ± 0.60	1.38 ± 0.22
Survival (%)	93.33 ± 5.77	100.00 ± 0.00	85.00 ± 21.21	90.00 ± 10.00
Biomass gain (g)	48.56 ± 8.40	44.84 ± 12.52	32.34 ± 21.60	39.97 ± 12.62

Mean (n=3 ± S.D.)

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Results

In the experiment, there was no significant difference in the growth parameters among treatments. The high values of the deviation are due to the high mortality in one of the replicates.

Discussion

The results obtained by Suryaningrum et al., (2020) suggest that up to 10% of the inclusion of macroalgae in the feed is beneficial to the animal, greater inclusions can interfere with zootechnical performance due to low digestibility. In this study, the treatment with the highest inclusion (15%) was not significantly different from the others. In previous studies, an increase in protein of the macroalgae *Ulva lactuca* was observed when cultivated in an integrated system with bioflocs, with values of 22.4% compared to cultivation in a laboratory solution of 12.4% protein (Chagas, 2021). The use of this biomass can generate better results in the digestibility of the feed and reduce or not use ingredients such as wheat flour. The similarity of the initial weight of the fish may cause an increase in aggressive behaviours during the experiment (Boscolo et al., 2011), which may explain, in some cases, the mortality rate.

Conclusion

Macroalgae meal, that up to 15%, can be used in Nile tilapia diets without interfering with the zootechnical performance and replacing ingredients.

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EUROPEAN EEL LARVICULTURE:
FIRST ESTABLISHMENT OF FEEDING LEPTOCEPHALUS CULTURE

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Introduction

Anguillid eels are targeted, high-value species for aquaculture in Asia and Europe. Unfortunately, eel farming is still capture-based, exclusively relying on wild-caught glass eels and thus, the sustainability of this industry is challenged by the present critically low stock abundance, which is especially the case for European eel, *A. anguilla* (Pike et al., 2020). Hence, it is urgently needed to further develop and establish captive breeding techniques and technologies for this critically endangered fish species. Advances in Japanese eel culture (Okamura et al., 2013) have formed the baseline for eel research, promoting recent development of hatchery protocols for European eel (Tomkiewicz et al., 2019). While offspring culture techniques for the European eel encompass the endogenous feeding stages (Sørensen et al., 2016), the transition to exogenous feeding is still challenged by lack of insights regarding the feeding stages and dietary requirements for the unique leptocephalus larvae. Research has been conducted to identify natural eel larval feeding sources (Miller, 2009) and physiology (Knutsen et al., 2021), but despite increasing knowledge on larval feeding ecology, the natural first-feeding regimes of *Anguillid* pre-leptocephali remain an enigma. Scientific inquiry has focused on identifying potential first-feeding diets with the first exogenously feeding experiments of hatchery-reared European eel larvae only recently attempted (Butts et al., 2016; Politis et al., 2018), in combination with enhanced rearing techniques aiming at improving larval survival (Tomkiewicz et al. 2019; Politis et al., 2021). In continuation of this work, research in the innovation project ITS-EEL has focused on developing prototype diets, exploring feeding and gut-priming regimes as well as testing larviculture procedures by applying progressively advancing culture techniques and technology. The presentation will overview these progressive advancements regarding the requirements of larval European eel in terms of abiotic (such as temperature, salinity, light, pH) and biotic (such as gut-priming, feeds, feed amounts, feeding regimes, microbial control, water quality) factors, from a morphological and molecular point of view.

Material and methods

European eel gametes were obtained through assisted reproduction using routine procedures as previously described by Kottmann et al. (2020). After fertilization, the floating layer was transferred to 60 L black conical incubators, supplied with conditioned filtered seawater (Politis et al., 2018) at a flow through rate of ~350 mL min^{-1}, while gentle aeration was added. Temperature was set to 18°C, salinity to 36 psu and light to low intensity of ~10 lx for better embryonic development (reviewed in Tomkiewicz et al., 2019). Larvae hatched at ~56 hpf, which were then reared in ~80 L tanks, connected to a recirculating Aquaculture System (RAS), until reaching the feeding stage. Thereafter, larvae were moved into ~8 L Kreisel tanks, connected to a new RAS, where temperature was kept at ~20°C and salinity lowered to ~18 psu (Politis et al., 2021). Here, larvae were fed prototype liquid diets, provided to the bottom of each tank. During feeding, water flow was stopped and lights turned on at high intensity of ~20 µmol m^{-2} s^{-1}. After ~30 min feeding time, the remaining food was flushed away, water circulation was restarted and the light turned off again. Larvae were fed using this procedure five times per day.

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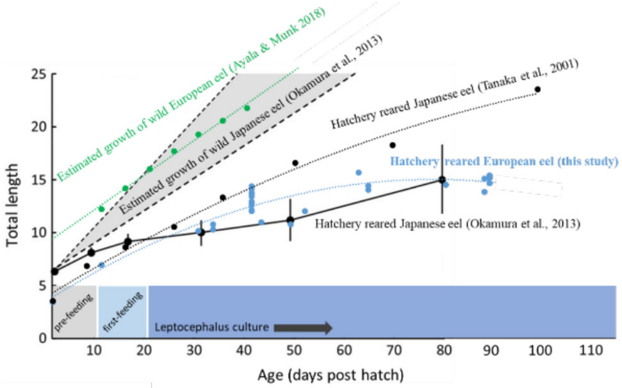

Fig. 1: Length of hatchery reared & wild European and Japanese eel offspring

Results and Discussion

Following constantly updated assisted reproduction protocols, the amount and quality of European eel larvae reaching the first-feeding stage has steadily increased enabling research efforts around larval feeding culture. Knowledge about larval European eel requirements and preferences, regaridng abiotic (temperature, salinity, light, pH) and biotic (gut-priming, feeds, feed amounts, feeding regimes, microbial control, water quality) factors has enhanced survival. Moreover, the European eel larval rearing systems and diets have been progressively upgraded and refined by incrementally integrating gained knowledge, resulting in the first ever European eel leptocephalus culture, surviving up to ~140 dph. Notably, European eel larvae grew from ~3 mm (~1.5 mm^2) at hatch, to 7-8 mm (3-4 mm^2) at the first-feeding stage (10 dph). The majority of larvae started feeding at 10-12 dph, while the larvae that did not feed or start feeding too late, entered the so called "point-of-no-return" and perished at 20-24 dph. In contrast, larvae that initiated exogenous feeding success-fully, survived beyond this point and transformed into the characteristic leaf-like leptocephalus shape. So far, applying advanced rearing and feeding procedures have led to European eel larvae growing to a length of ~15 mm and body area up to ~20 mm^2. These results are comparable to Japanese eel larviculture (Tanaka et al., 2001; Okamura et al., 2013), while growth rates are lower compared to estimate patterns for wild leptocephali (Fig. 1). Overall, our results, enabling the first ever European eel leptocephalus culture, present a new promising step towards closing the life cycle of this critically endangered species in captivity. Now, the challenges ahead involve progression of diets, advancement of rearing techniques and improvement of larval survival throughout the leptocephalus stage, to reach the glass-eel stage.

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EFFECTS OF DIFFERENT DIETS ON THE SURVIVAL AND GROWTH OF THE SEA CUCUMBER *Holothuria arguinensis* HATCHERY-REARED JUVENILES

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The growing demand for sea cucumber products constitutes a continuing threat to wild stocks and to the habitats in which they live in. A possible way to mitigate the detrimental effects economic demand has on ecosystems around the world is to increase supply, as such, aquaculture stands as an emerging alternative. A promising species for future European aquaculture production, with high commercial interest, is the sea cucumber *Holothuria arguinensis*. With a natural distribution along the North-Eastern Atlantic, including the coast of Portugal, this species is already showing a decrease in wild populations due to unregulated exploitation. With the implementation of successful larval rearing and settlement, more emphasis needs to be placed on the on-growing phase of juveniles. The optimisation of the diets and feeding of *H. arguinensis* juveniles to reduce their mortality and increase growth performance has not yet been studied. This species feeds on the sediment and studies with other detritivores species point to the inclusion of macroalgae as part of the diet. In this regard, the following study aimed to assess growth and survival rate of hatchery-reared *H. arguinensis* juveniles (n=324; mean weight: 20±7 mg; mean length: ≈ 1 mm). Individuals were distributed in 3 RAS, each with three 50 L tanks with sandy substrate and were fed during 6 months with three diets (Diet 1 = *Rhodomonas baltica* + *Chaetoceros calcitrans*; Diet 2 = *Rhodomonas baltica* + *Saccorhiza poliscides* and Diet 3 = *Rhodomonas baltica* + *Chaetoceros calcitrans* + *Saccorhiza poliscides*) administered 3 times a week. *H. arguinensis* fed with diet 2 showed the highest survival rate (84.26±14.25%) when compared with individuals fed with diet 1 (59.26±12.83%) and diet 3 (40.74±21.03%). Juveniles fed with diet 2 and diet 3 showed a significantly higher final weight (diet 2: 570±150 mg and diet 3: 480±50 mg) and a higher length (diet 2: 19±4 mm and diet 3: 17±9 mm) than individuals fed with diet 1 (270±80 mg and 11±2 mm). Specific growth rate (SGR) was higher in juveniles fed with diet 2 (9.23 % month^{-1}) than in juveniles fed with the diet 1 (4.11 % month^{-1}) or with the diet 3 (7.60% month^{-1}). The addition of the macroalgae *S. poliscides* in the *H. arguinensis* juvenile diet shows potential benefits on growth. At the end of the trial juveniles whose diet was supplied with *S. poliscides* showed an increased growth observed with a higher final weight, length and SGR when compared to individuals without this macroalgae in their diet. Results were most promising particularly with diet 2 where there was a higher survival rate. To optimize sea cucumber *H. arguinensis* rearing more trials should be conducted with hatchery-reared juveniles comparing sediment enrichments, the efficacy between macroalgae diets, the production with fishfarm waste or the growth in integrated multi-trophic aquaculture systems (IMTA).

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REPRODUCTIVE CYCLE OF THE INVASIVE ASCIDIAN *Styela plicata* (Lesueur, 1823) IN MUSSEL *Mytilus edulis* Linnaeus, 1758 AQUACULTURE

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Mussel farming has faced challenges such as the establishment of non-indigenous species that may become invasive (NIS) in the farming environment. These introductions often occur at an accelerated rate and fouling NIS can have a relevant negative impact in mussel production, such as mechanical interference with the shell function and competition for food and space. The presence of biofouling in marine aquaculture represents one of the main barriers to an efficient and sustainable production and its control is a challenge for which solutions are still being investigated. Some NIS, such as tunicates, cause significant negative effects on mussel productivity, by reducing the mussels' growth rates and causing severe mortality of the reared mussels. The tunicate *Styela plicata* is a widely distributed, temperate to subtropical, biofouling ascidian, which is associated with damages caused in aquaculture. This study focused on the reproductive biology of *S. plicata* present in mussel cultures in the Albufeira coastal lagoon (SW Portugal), aiming at contributing to a better understanding of how environmental variables such as temperature and salinity can influence its establishment and development. The reproductive cycle of *S. plicata* was characterized through the evaluation of the gonadosomatic index (GI). The histological analysis of the gonads was performed monthly (n=30) over an 18-month period to identify the gametogenic stages of the gonads of individuals collected from a mussel farm raft located in the Albufeira coastal lagoon. GI was highest in summer when the highest temperatures and salinities were also recorded. No clear seasonal reproductive pattern was observed since mature gametes were present throughout the year. Spawning can occur continuously, although more pronounced in late summer and early autumn. The present study confirmed the occurrence of an established population of *S. plicata* in the Albufeira coastal lagoon since the individuals analysed were fertile all year-round. A prolonged reproductive period can confer *S. plicata* a competitive advantage, favouring its permanence in areas that have already been invaded and offering the opportunity to expand its area of occurrence. Moreover, the temperature and salinity of the lagoon do not exceed the tolerance limits for the reproduction of the species and the low hydrodynamics favours the survival and the successful establishment of this tunicate. Therefore, it can be concluded that the current environment of the Albufeira lagoon offers advantages for *S. plicata*. These results suggest that additional studies on the dynamics of this population are needed, especially to clearly identify the time of greatest larval settlement and help to put in place actions to mitigate the adverse effects of this biofouling ascidian on mussel farming.

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PHYSICO-CHEMICAL WATER ANALYSIS AT THREE DIFFERENT DEPTHS FOR POTENCIAL OFFSHORE FISH FARMING IN PORTUGAL

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Introduction

Inappropriate water temperature and other physico-chemical water parameters can cause slow growth, induce stress or can even cause mortality, making the aquaculture less efficient. Considering that there is space on the Portuguese coast that could be used for offshore aquaculture, in this work, we intend to compare some physico-chemical water parameter values on the water column (up to 50 metres) with the ideal growth parameters of some fish species with economic interest and potential to be produced in offshore aquaculture.

Materials and methods

This study was conducted with field surveys on the Portuguese Coast in 5 areas (Area A: between Viana do Castelo and Oporto; Area B: between Aveiro and Figueira da Foz; Area C: Peniche; Area D: between Setúbal and Sines; Area E: Algarve region) and 4 different periods: October, 2018; April, 2019; October, 2019 and March, 2020. Temperature (ºC), salinity, pH, dissolved oxygen (mg-O_2 L^{-1}), total oxidized nitrogen (also known as nitrate+nitrite, μmol L^{-1}; NO_x^-) and ammonia (μmol L^{-1}; NH_3) were analysed in 3 different depths (5 m, 25 m and 50 m). Also, the mean monthly temperature data were collected from the NASA GHRSST (Group for High Resolution Sea Surface Temperature, Level 4) platform to evaluate the surface monthly temperatures in the studied areas. The optimal and maximum and minimum limits of the previous parameters tolerated by some potential fish species (*e.g. Argyrosomus regius*, *Sparus aurata*, *Dicentrarchus labrax, Seriola dumerili, Salmo salar* and *Sardina pilchardus*) that can be produced in these locations were compiled and these data were compared with the physico-chemical water parameters data determined in each field survey, area and depth.

Results

During the field surveys of 2018, 2019 and 2020, the parameter that changed significantly throughout the water column was the temperature. Overall, the area that presented the most extreme mean temperatures were area E, with a mean maximum of 22.75 ºC in the 2018 survey at 5 m deep, and area A, with a mean minimum of 13.38 ºC in the October 2019 survey at 50 m deep. There have been some variations of pH, dissolved oxygen and salinity values in the water column. The mean values of pH varied from 7.93 (area C; 5 m; October, 2019) to 8.14 (area D; 5 m; April, 2019); dissolved oxygen mean values varied from 6.43 (area A; 50 m; October, 2019) to 9.26 (area A; 5 m; April, 2019) and salinity mean values varied from 35.35 (area A; 5 m; April, 2019) to 36.71 (area E; 5 m; October, 2018). Total oxidized nitrogen, nitrites and ammonia values were consistently below the respective limits of quantification. All parameters analysed are within limits tolerated by the potential species that can be produced except temperature.

Discussion

Comparing the ideal temperature growth values of the most economical interesting species that can be produced in mainland Portugal it is possible to highlight the areas where it is achievable to implement submerged sea cages for each species. The meagre *Argyrosomus regius* can withstand a wide range of temperature values (13 – 29 °C) with optimal values ranging from 24 ºC to 29 ºC (Stavrakidis-Zachou et al., 2021) and can be produced in south of Portugal to depths up to 50 m. The gilthead sea bream *Sparus aurata* and the sea bass *Dicentrarchus labrax* have similar ideal growth values between 20 ºC and 25 ºC (Zhang *et al.,* 2021) which make them species that can also be considered for deep farming in southern Portugal. For the greater amberjack *Seriola dumerili* the information available suggests that this species has an optimal growth at warmer temperatures and the southern area would also be the most suitable. On the other hand, salmon *Salmo salar* needs lower temperature being able to withstand values from 14 ºC to 18 ºC (Johansson *et al.,* 2009) that makes it potentially promising for the central and northern area of Portugal. For new species under investigation that are not yet commercially produced as sardine *Sardina pilchardus* the information available is scarce and more studies are needed to understand which areas would be the most promising for its rearing. Submerged sea cage aquaculture is an intricate process that has multiple variables, such as feeding routines, monitoring, storm prevention structures, net material, currents, depth deployment and anchor weights that need to be considered. Nevertheless, there is potential to insert new depth structures in Portugal but further studies are needed to reduce the risks associated with its implementation.

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ASSESSING THE ROLE OF ULVAN EXTRACTS AS FUNCTIONAL INGREDIENTS IN AQUACULTURE

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Introduction

Aquaculture industry is one of the fastest growing food production sectors becoming generalized intensive and semi-intensive practices and therefore, increasing the susceptibility to infectious diseases. The use of antibiotics has been generalized, not only as therapeutic measure to control bacterial pathogens but also as growth promoters despite the side effects that these practices involve (Lulijwa et al., 2020). For this reason, the scientific community has focus on the research of bioactive compounds with immunostimulatory properties and be able to conduct preventive strategies. Ulvans are marine derived polysaccharides extracted from green macroalgae of the genus *Ulva* that possess immunomodulating, antioxidant, anticoagulant, anticancer, antiviral and antihyperlipidemic activities (reviewed in (Kidgell et al., 2019)). Recently, the role of ulvan extracted from *Ulva ohnoi* as bioactive compound with immunomodulatory activity in *Solea senegalensis*, a species of great interest in aquaculture, has been reported (Ponce et al., 2020). However, the purification grade is an important issue to consider as well as the administration method. The aim of this study was to investigate the effects on the immune system of *S. senegalensis* fed diets with the inclusion of the functional ingredient ulvan. For this reason, the influence of the purification grade on the structure and chemical composition of ulvan extracts and therefore, on their biological activities throughout *in vitro* assays in *S. senegalensis* macrophages was evaluated. Thereafter, *S. senegalensis* juveniles were fed diets with the selected ulvan extract with potential properties to enhance the defence system at ratios 0, 1 and 2% for 30 days. Finally, juveniles were challenged with *Photobacterium damselae* subsp. *piscicida*, an important fish pathogen, and the mortality rate was determined.

Material and methods

Macroalgae *U. ohnoi* was supplied by Huerta Marina Huelva S.L. (Spain). The sulfated polysaccharide ulvan was extracted following the method described by (Fernández-Díaz et al., 2017) without the use of sodium oxalate during the extract process. Three ulvan extracts with different purification grade were obtained referred as: CUE (crude ulvan extract); UE (precipitation of ulvan with ethanol) and UU (ulvan obtained by ultrafiltration). For *in vitro* assays, macrophages from head kidney of *S. senegalensis* juveniles were extracted and cultured as described by Secombes and Fletcher, (1992). A total of 40 juveniles (average weight 85.63 ± 24.91 gr) were obtained from facilities of IFAPA Centro El Toruño (El Puerto de Santa María, Spain) and head kidney was sampled under aseptic conditions. The cell suspension was loaded on a 34%51% discontinuous Percoll (Sigma-Aldrich) gradient. Cells were distributed into 24-well cell culture plates at 2 x 10^6 cells ml^{-1}. After 24 h of incubation with ulvan extracts (0.05, 0.5 y 1 mg ml^{-1}), cells were collected. Three replicates were used per treatment and the experiments were performed three times. Total RNA was isolated using the RNeasy® Mini Kit (Qiagen) and treated twice with DNase I using the RNase-Free DNase kit (Qiagen). Total RNA was reverse transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad). Expression analyses of a set of genes related to the antioxidant and immune system were performed by RT-qPCR using specific primers. For *in vivo* assays, *S. senegalensis* juveniles (average weight 33.16 ± 6.64 gr) were provided by Cupimar S.A. (Cádiz, Spain) and they were acclimated for 14 days. Three groups of fish in triplicate were fed the diets with UE at rates of 0, 1 and 2% for 30 days. The feeds were developed by LifeBioencapsulation S.L. (Almería, Spain). At 30 days post treatment, spleen was sampled, transferred to RNAlater™ Stabilization Solution (Invitrogen) and store at -80ºC until use. Expression analyses were performed as described above. Blood was drawn from the caudal vein, plasma was collected and stored at -20ºC until further análisis. Lysozyme Detection Kit (Sigma-Aldrich) was used to determine the lysozyme activity in plasma. *S. senegalensis* juveniles were challenged with the strain Lg41/01 of *Phdp* and mortality was monitored daily.

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Results and discussion

In order to investigate the effects of the purification grade on the biological activities of ulvan, the expression level of a panel of genes related to the antioxidant and immune system was evaluated. The selected genes were as follows: superoxide dismutase 1 (*sod1*), catalase (*cat*), glutathione peroxidase 1 (*gpx1*), thioredoxin (*txn*), lysozyme g (*lyg*), interferon regulatory factor 1 (*irf1*), interleukin 1b (*il1b*), interleukin 6 (*il6*), interleukin 10 (*il10*), complement C3 (*c3*) and complement C7 (*c7*). In relation to the expression pattern of genes related to the antioxidant system, no significant differences in the expression levels of *gpx1* and *cat* were observed in any case. Expression levels of *txn* were significantly higher in the case of CUE and UU treated macrophages than in the control groups. However, mRNA levels of *sod1* were only significantly higher in the case of CUE treated macrophages. Regarding genes related to the immune system, only the mRNA level of *c7* was significantly higher in macrophages treated with CUE extracts than in the control groups. Nevertheless, a significant coordinate increase in the mRNA levels of *lyg*, *il1b*, *il6*, *il10* and *c7* were observed in macrophages incubated with both, UE and UU extracts with respect to the controls at concentrations of 1 mg ml^{-1}. Based on these results, UE extract was selected to be incorporated into the functional feeds for the evaluation of the effects in the immune system of Senegalese sole juveniles. All the results obtained in this study will be presented and discussed in depth during the oral presentation.

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EFFECTS OF THE EXTRACTION AND PURIFICATION METHODS ON THE BIOACTIVITY OF ULVAN

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Introduction

Ulvan is a marine sulphated polysaccharide obtained from green seaweeds of the genus *Ulva* that has a wide range of bioactivities including immunomodulating, antioxidant, anticoagulant, anticancer, antiviral and antihyperlipidemic activities (Reviewed in Kidgell et al. (2019)). Proposed methods to extract ulvan include hot water extraction, hot water with sodium oxalate, acidified ammonium oxalate or hydrochloric acid, among others. The extract is separated from the residual biomass by filtration and/or centrifugation being the isolation of ulvan performed by precipitation, evaporation or concentration by ultrafiltration followed by lyophilisation. Extraction conditions as well as the purification methods influence the composition of the extracted ulvan and thereafter, their biological activities (Wahlström et al., 2020). Further research is needed to have a deep understanding of the general trends in physicochemical properties and biological activities of ulvan relative to its structural features. The aim of this study was to assess the effects of the extraction method of ulvan and the purification grade on their structural and biological properties. Acidic and hot water extraction methods were compared as well as the isolation and purification grade of the extracted ulvan. The antioxidant and immunostimulant activities and the protective effect induced by the different types of ulvan on *S. senegalensis* macrophages were evaluated.

Material and methods

Macroalgae *U. ohnoi* was supplied by Huerta Marina Huelva S.L. (Spain). The sulfated polysaccharide ulvan was extracted following two methods: one extraction with hydrochloric acid (HCl) as described by Yaich et al. (2014) and another one by an aqueous extraction protocol as described by Fernández-Díaz et al. (2017) but without the use of sodium oxalate. Crude ulvan extracts were referred as CUE-HCl and CUE-H_2O, respectively. From each extraction method, ulvan were obtained by ultrafiltration and they were named as UU-HCl and UU-H_2O, respectively. Antioxidant activity of ulvan was evaluated by determining their DPPH and superoxide anion radicals scavenging abilities according to (Chen et al., 2016). Macrophages from head kidney of 30 juveniles *S. senegalensis* juveniles (average weight 88.85 ± 27.41 gr) obtained from facilities of IFAPA Centro El Toruño (El Puerto de Santa María, Spain) were extracted and cultured as described by Secombes and Fletcher, (1992). After 24 h of incubation with ulvan extracts (0.05, 0.5 y 1 mg ml^{-1}), cells were collected. For the H_2O_2 challenge, macrophages were isolated from 36 Senegalese sole juveniles (average weight 74.16 ± 9.30 gr) and treated with ulvan extracts as described before. Afterwards, the medium was removed and new medium containing 1 mM H_2O_2 was added and cultivated for another 1 h. Three replicates were used per treatment and the experiments were performed three times. Total RNA was isolated using the RNeasy® Mini Kit (Qiagen) and total RNA was reverse transcribed using the iScript^{TM} cDNA Synthesis kit (Bio-Rad). Expression analyses of a set of genes related to the antioxidant and immune system were performed by RT-qPCR.

Results and discussion

The scavenging rate on superoxide anion radical of ulvan obtained after aqueous extraction was 9.73-fold higher than that of ulvan obtained from HCl extraction. All of them showed lower scavenging activity than ascorbic acid (AA). No significant differences were observed between DPPH scavenging rates being 12.70, 12.22, 13.39 and 13.59 % respect to the AA for CUE-HCl, CUE-H_2O, UU-HCl and UU-H_2O extracts, respectively. To deep investigate the effects of both, the extraction method and the purification grade of the ulvan extracts on the biological activities, the expression level of a panel of genes related to the antioxidant and immune system was evaluated. The selected genes were as follows: superoxide dismutase 1 (*sod1*), catalase (*cat*), glutathione peroxidase 1 (*gpx1*), thioredoxin (*txn*), lysozyme g (*lyg*), interleukin 1b (*il1b*), interleukin 6 (*il6*) and interleukin 10 (*il10*). In relation to the expression pattern of genes related to the antioxidant system, no significant differences in the expression levels of *cat* were observed in any case. Expression levels of *gpx1* were significantly lower in the case of UU-HCl and UU-H_2O treated macrophages than in the control groups, whereas no significant differences were observed between crude extracts treated groups. In the case of *sod1*, mRNA levels were only significantly higher in the

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case of both CUE treated macrophages. Expression levels of *txn* were significantly higher in the case of all ulvan extracts treated macrophages than in the control groups at concentrations of 1 mg ml^{-1}. Regarding genes related to the immune system, a significant increase in *lyg*, *il1b*, *il6* and *il10* mRNA levels were observed only in UU-H_2O treated macrophages at concentrations of 1 mg ml. In order to further investigate the antioxidant properties of the ulvan extracts, a H_2O_2-induced injury cell model was performed, and gene expression patterns were evaluated. A significant decrease in mRNA levels of *sod1* and *gpx1* were observed in H_2O_2 treated macrophages with respect to the control groups whereas no significant differences were observed in the case of macrophages preincubated with the four types of ulvan. Changes in the structure of ulvan during the extraction procedure may reduce or enhance its functionality. These results indicate that the biological functions of CUE and UU ulvan are more related to antioxidant and immunomodulating activities, respectively.

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ALLOCATED ZONES FOR AQUACULTURE (AZAs) IDENTIFICATION IN THREE AQUATIC ECOSYSTEMS: SEA, LAGOONS AND LAKES

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Introduction

Aquaculture industry is a strategic development sector of the blue economies, considering the huge amount of seafood consumption in the EU, but only 20% comes from EU farms (EUMOFA, 2018). Indeed, the sector is stagnating due to different problems, such as administrative, environmental, and technical criticalities. In order to promote a sustainable, innovative and competitive aquaculture, a spatial planning should be carried out to highlight the most suitable areas to this activity. In this framework the identification of the Allocated Zones for Aquaculture (AZAs), areas where aquaculture has priorities over other uses (Sanchez-Jerez et al., 2016), can booster this sector. An AZA is a planning system that considering the environmental, social, and economic components, allowing to highlight the most suitable areas to specific farming activities.

In 2020, in Sardinia, started a project about the AZA identification (EMFF PO 2014-2020), with 3 main objectives: (i) improve the aquatic ecosystems knowledge; (ii) prepare the AZA regional plan; (iii) develop a Geoportal for managing and consulting data. Based on the regional resolution (Sardinian Region resolution 3/26 of 22/01/2020), the AZA have to be classified as follow: ZONE 1, areas suitable for aquaculture activities; ZONE 2, areas suitable for aquaculture activities subjected to regulation/limitation; ZONE 3, areas not suitable for aquaculture activities.

In this paper we present a framework for supporting the AZA identification in three aquatic ecosystems: at sea, in lagoons, and in lakes.

Methods

The AZA plan pertained to the Sardinian territorial sea waters (SW), lagoons (LG) and lakes (LK). A tiered approach was developed that first assessed the availability of the three aquatic ecosystems for aquaculture. Following this, in the areas considered to have good availability for aquaculture, the suitability for different species was estimated. In these ecosystems data about chemical-physical water characteristics, habitat distribution, and current uses were collected. Data were extracted from regional agencies database (*in situ* data), remote sensing database (*i.e.*: CMEMS), and cartographic data gathered from different sources (e.g.: Sardinian Geoportal and EMODnet).

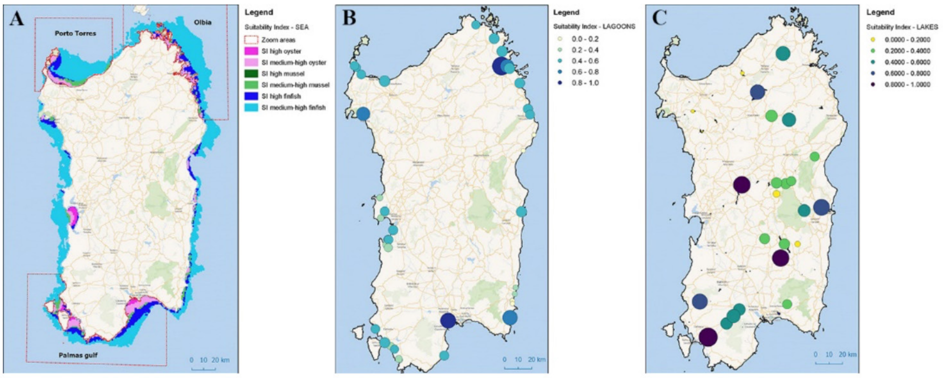

Figure 1. Aquaculture suitability index (SI) estimated for the three aquatic ecosystems: sea (A), lagoons (B) and lakes (C).

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In the three aquatic ecosystems, the species considered were: European seabass (SW, LG), gilthead seabream (SW, LG), Mediterranean mussel (SE, LG), Pacific oyster (SW, LG), flathead grey mullet (LG), grooved carpet shell (LG), rainbow trout (LK), brown trout (LK), carp (LK), perch (LK), tench (LK), largemouth bass, and striped bass (LK).

At sea, the AZA were identified applying the Spatial Multi-Criteria Evaluation methodology (Brigolin et al., 2017; Porporato et al., 2020), considering 4 criteria: potential growth, distance to harbour, significant wave height, bottom typology. Protected habitats distribution, sea uses, and potential conflicts, with their specific buffer zone, were also considered. Subsequently, in 3 different zoom areas, namely Porto Torres, Olbia, and Palmas gulf (Fig. 1A), a dispersion index for finfish farms was estimated. Finally, the Cumulative Effect Assessment and the Maritime conflict analysis tools, based on the Tools4MSP Modelling Framework (Menegon et al., 2018), were applied in the Porto Torres area.

In lagoons and lakes, firstly the aquaculture suitability was estimated combining 2 methodologies, the fuzzy logic classification and the factorial analysis. In these systems biological (*e.g.*: temperature, oxygen) and logistic criteria (*e.g.*: site accessibility and facilities) were considered. Subsequently, depending on these first results, in depth analyses in 3 lagoons and in 2 lakes were carried out. Pacific oyster carrying capacity in the three lagoons was calculated using data on water movement and food availability. In the lakes, the suitability of Sardinian reservoirs for rainbow trout aquaculture was evaluated through seasonal field samples of temperature and oxygen at different locations and depths.

In parallel to the previous described analyses, in order to draft a participatory AZA plan, and to select the zoom areas, different stakeholders, including local authorities, farmers, the scientific community, and the general public, were involved in different events.

Results and discussion

The main results of the aquaculture suitability estimated for the 3 aquatic ecosystems are show in Fig. 1. A Geoportal based on GeoNode, containing data and tools was developed (www.waterspatialplanning.eu). Our research was aimed at identifying the environmental, social, and economic conditions that allow the development of a sustainable aquaculture, guaranteeing the environmental protection, and the reduction of potential conflicts between different uses, facilitating synergies and co-uses.

The obtained results will be used to draft the AZA Sardinian plan which will be instituted by the competent authority. Spatial planning and the choice of areas has been based on the use of scientific, environmental, legal, technical and socio-economic evidence, to guarantee the conservation and protection of the environment and territories, maintaining a good ecological status, and preventing habitat and ecosystem degradation.
Our approach, thanks to the generality and transferability of the applied methodology could be applied to the AZAs identification in other case studies.

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STAKEHOLDERS INVOLVMENT IN THE ALLOCATED ZONES FOR AQUACULTURE (AZAs) IDENTIFICATION PROCESS: THE SARDINIAN EXPERIENCE

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Introduction

The aquaculture development needs to improve spatial planning process in order to ensure socio-economic sustainability, effective preservation of protected habitats, food security and employment generation. The Allocated Zones for Aquaculture (AZAs; Sanchez-Jerez et al., 2016) identification should be carried out in synergy with all the stakeholders through a participatory approach in the planning process (Corner et al., 2020). Indeed, aquaculture growth depends on a complex value chain influenced by different societal and governmental factors that can determine the successful, or the failure, of the AZA identification and planning. Moreover, multiple uses and users of spaces, including coastline and lagoons, can coexist or, frequently, have conflicting interests and needs.

In 2020, in Sardinia, started a project about the AZA identification (EMFF PO 2014-2020), which since the beginning has foreseen the direct involvement of different stakeholders, including the decision makers, farmers, fishers, researchers and academics, and the general public. In this paper we present the results of stakeholders' involvement in the AZA identification carried out in Sardinia (Italy).

Methods

Two online meetings were organised, one restricted with the decision makers, public institute and agencies, and one public. In the first meeting were involved 4 regional departments, 2 regional agencies, and the institute responsible for the veterinary and public health. The second meeting involved the general public, farmers, fishers and researchers and academics.

Subsequently, in order to define the current state of aquaculture in Sardinia, collect necessary data to estimate the suitability, and to stimulate the involvement of some stakeholders' categories, semi-structured questionnaires were produced and distributed to all the farmers operating in Sardinia, both in lagoons and at sea. The structure of the questionnaire was conceived in four sections: (i) characteristics of the farming site, services and facilities present, (ii) species reared and breeding cycle, (iii) economic information, (iv) environmental pressures.

Results and discussion

During the two online meetings we collected few feedbacks about the AZAs identification, both by the decision makers, farmers and the general public which have not a proactive involvement. The interactive involvement was established with the researchers and academics which asked details about the process, but not giving advices about the selected areas. In our opinion, the participation and direct involvement of the stakeholders was affected by the COVID-19 restriction. Indeed, the meeting held online and not in presence not allowing us to involve properly and in a more active way the stakeholders.

Concerning the questionnaires, 39 responses from farmers, operating both at sea and in the lagoons, were collected. The farming site characteristics, services and facilities highlighted that the majority of farms have different facilities specific for aquaculture activities, such as rearing tanks, equipment for sorting and packaging live bivalves, and enclosed spaces in the lagoons. The farmed species resulted mainly seabream (64%), seabass (59%), mullets (49%), mussels (33%), oysters (33%), and clams (33%). Concerning the economic section, the results highlighted a varied situation, with a total annual turnover that exceeds 65 million euros, of which more than half, however, derives from a single cooperative. The marketing channels are linked to wholesaler (96.9%), direct sales (43.8%) and retail sales (37.5%). As regards the destination of the product, 35.9% is commercialised at local level, 43.6% regional level and 28.2% at national level.

Regarding the last section of the questionnaire, the environmental pressures at the farmer sites perceived by the farmers themselves, the results are reported in the figure 1. As one can see, the most frequent pressure is related with the damage from fish-eating birds (*i.e.*: cormorants).

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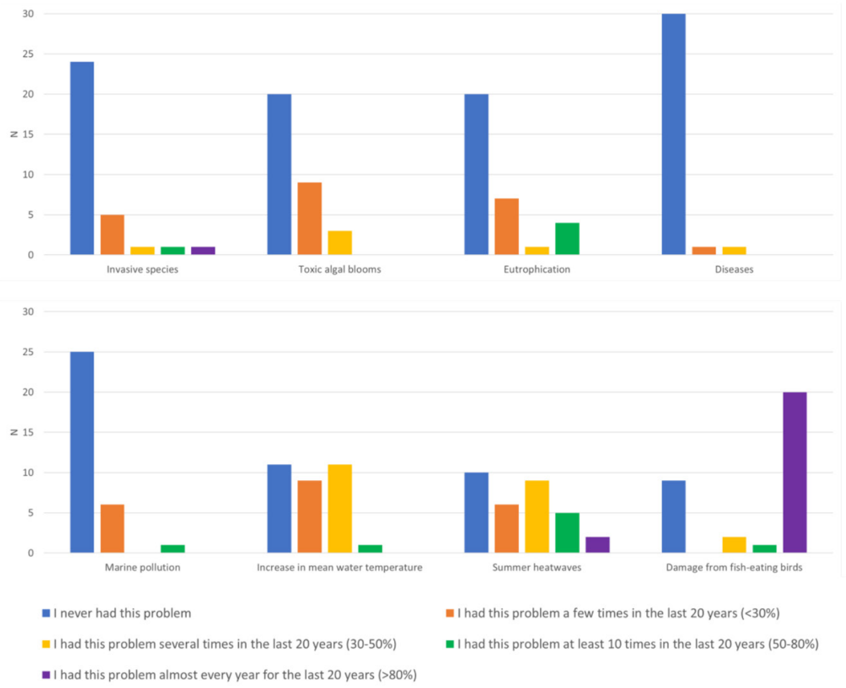

Figure 1. Frequency of the environmental pressures in the farming sites perceived by the farmers.

The results of the stakeholders' involvement in the AZA identification process underlined the initial reluctance within the participatory process, related also to the online meetings. In the next months, different strategical events in presence will be organised in order to present the draft of the AZA plan for the Sardinian region, and to inform properly the stakeholders about the project outputs, as well as asking again possibile useful feedbacks and suggestions for the AZA plan implementation.

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AN EMERGENCY CALL TO DEVELOP "ACTION PLAN FOR CONSERVATION OF STURGEON FROM THE CASPIAN SEA"

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The Caspian Sea was the main basin for the world sturgeon resources in 3 decade ago, where produce more than 28000 tons of sturgeon meat and 3000 tons of caviar. Illegal catch, poaching, destruction of living or spawning sites, pollutions and lack of appropriate coordinated management of Caspian littoral states for unique shared stocks were the main reasons to lose sturgeon species to close to extinct level. Despite having a zero quota for commercial catch and export to international market, based on CITES agreement all sturgeon catch should allocated for rehabilitation and restocking as well as for research purposes. The objective of this paper is to demonstrate the critical situation of sturgeon resources in the Caspian Sea and call for collaboration, sharing the knowledge and experience to save the sturgeon in the Caspian basin before this unique species become extinct.

Since 1997, The Convention on International Trade in Endangered Species of Wild fauna and Flora (CITES), beginning from COP-10 to COP 14, tried to save the wild sturgeons resources by adopting several decision and resolution to allocate the catch and export quota for the Caspian Sea and all sturgeon basin, however no success were achieved for sturgeon conservation. It clearly demonstrated that sturgeon conservation and sustainable use can be solved by appropriate management of shared stocks. In other words regulation the catch or banning of international trade will only shift the market from international to national or regional trade!

In recent years in 2020 and 2021, the lowest number of sturgeon brood stocks were cached, other crises such as the climate change, shortage of rainfall and running water in rivers and remains very limited area for natural spawning caused that natural and artificial spawning reached to its minimum level close to zero for some sturgeon species. Totally less than 10 specimens of Ship (*Acipener nudiventris*) and Beluga (*Huso huso*) were cached last year and getting lower and lower by years.

Since 2011 a self-moratorium of sturgeon commercial catch and caviar export was established by the Caspian littoral states, unfortunately no progress have been observed on the status of sturgeon stock resources. It indicates that still illegal catch is on the place and market shifted mostly into the domestic level. As it proposed by CITES, the Caspian Sea littoral states should conduct a reliable stock assessment and share the information in order to evaluate the impact of fishing ban and trade control!. At present before the complete extinct of rare sturgeon an establishment of sturgeon live gene bank is necessary in order to develop a genetic bases for conservation and restocking program as well as for aquaculture extension.

In conclusion, before it become late it is needed to act immediately by 5 Caspian Sea littoral states to develop an standard joint Action Plan similar to sturgeon action plan established in Europe, and North America sturgeon species. To support for such initiative it should call for national, regional and international communities to join in to this long term management program for sturgeon conservation in the Caspian Sea. It is clearly approved that development of Action Plan is the first steps towards the resource management, It need strong political wishes and financial support as well as all necessary tools to implement the Action Plan.

There are excellent experiences by FAO for the Sustainable management of shared resources, IUCN guideline for Action Plan as well as the World Sturgeon Conservation Society (WSCS) published Decelerations on global sturgeon conservation (Ramsar, 2005 and Vienna 2019).These documents can be used to develop appropriate Action Plan and its implementation.

LONG-TERM CHARACTERIZATION OF ENVIRONMENTAL CONDITIONS AND MICROBIAL POLLUTION IN SHELLFISH GROWING AREAS OF EMILIA-ROMAGNA, ITALY

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Introduction

Shellfish aquaculture is a vulnerable sector to climate change (Zgouridou et al., 2022). There is a clear evidence of climate change (e.g. temperature rise, extreme events) in the Emilia-Romagna region (AdriaClim, 2021) where 145 growing areas for shellfish are based (23% of total national shellfish areas). Monitoring of shellfish areas is routinely carried out by competent authorities to ensure environmental quality in transitional and marine waters and seafood safety.

In this study, historical dataset of *Escherichia coli* measured in harvested shellfish and growing waters of ER, were analyzed for cross correlations with environmental parameters and for implementation of a microbial diffusion model under climate change scenarios.

Materials and Methods

Field data collected between 2005 and 2021 from 24 nearshore stations in Emilia-Romagna have been analyzed for β-glucuronidase positive *Escherichia coli* (*E. coli*) in the shellfish and the growing waters (respectively ShellEcoli, WatEcoli). Statistical analysis has been carried out to characterize the frequency of noncompliance in the classified shellfish area and relationship with environmental conditions, focusing on water salinity (Sal), dissolved oxygen (O2), pH and surface temperature (T_w). Environmental data were collected *in situ* at the same shellfish sampling time. The frequencies have been calculated for ShellEcoli thresholds (MPN/100g) set by European Regulation 627/2019: 230 (class A, direct human consumption), 4.600 (class B, consumption after purification or relaying) and 46.000 (consumption after relaying over a long period) and WatEcoli (MPN/100ml).

The relationships between ShellEcoli and WatEcoli, and environmental parameters measured during official monitoring campaigns have been calculated. For each sampling station, temporal averages of ShellEcoli, WatEcoli, salinity, O2, pH and water temperature have been evaluated. Then, the cross-correlation coefficient (CC, aka Pearson correlation) has been calculated on the resulting set of data (temporal mean values for each station) to evaluate the relationship between ShellEcoli and WatEcoli and with respect to environmental parameters.

Results

The average of ShellEcoli at stations located in zone "A" is 79.97 MPN while it is 994.21 MPN for stations in zone "B". The maximum of ShellEcoli at various stations ranges between 1.300-9.200 MPN in zones "A" and 3500-54.200 MPN in zones "B". The mean percentage of measurements whose ShellEcoli exceeded 230, 4.600 and 46.000 MPN resulted 7%, 0% and 0% respectively in zones "A", and 42%, 5% and 0% respectively in zones "B".

Given WatEcoli, the average at stations in zone "A" is 1.1 MPN while it is 14.29 MPN for stations in zones "B". The maximum of WatEcoli at various sampling stations ranges between 48-230 MPN in zones "A" and 26-2.400 MPN in zones "B". Regarding CC values, the ShellEcoli and WatEcoli parameters do not show a significant CC (0.14).
A significant negative CC between ShellEcoli and salinity, O2 and pH (-0.71, -0.80 and -0.68 respectively) was found. While water temperature does not show a significant CC (-0.29). CC between WatEcoli and salinity, O2 and pH show similar results but with a weaker relationship (-0.72, -0.65 and -0.52 respectively), while water temperature does not show a significant CC (-0.15).

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Discussion and conclusion

Results proved that a robust classification procedure has been done in agreement with legal framework and limited noncompliance events were observed in shellfish A and B zones. Significant relationships between environmental (Sal, O2, pH) and microbial parameters were found. The absence of a clear correlation between WatEcoli and ShellEcoli may be related to a delay in the absorption of WatEcoli by shellfish. Data analysis also highlight the expected effect of salinity, dissolved oxygen and pH on *E. coli* concentration (both ShellEcoli and WatEcoli). The absence of CC between *E. coli* with temperature may be explained as even if WatEcoli is affected by environmental parameters, its concentration also depends on the actual quantity of *E. coli* discharged in the sea that is not constant. Hence, in order to carry out a clear analysis of the importance of the different environmental parameters it is necessary to follow *E. coli* pollution from its source of release. To overcome this challenge, historical dataset together with further near real-time *in situ* data will be used for the implementation of a microbial diffusion model. This represents an operative tool for regional authorities and industry sector to estimate the extension of shellfish growing areas potentially influenced by fecal pollution from point sources in present and climate change scenarios in order to develop adaptive strategies for the long-term sustainability of shellfish farming.

Acknowledgments

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VIBRIOS WITH PATHOGENIC POTENTIAL FOR MARINE AQUACULTURE IN PHYTOPLANKTON CULTURES IN A BATCH SYSTEM

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Introduction

Live feed is essential in marine aquaculture. Among the most important are the cultures, which are generally used in larval stages. Phytoplankton cultures have a significant bacterial load, including beneficial and necessary bacteria. Unfortunately, however, they can also harbour vibrios, including species potentially pathogenic to the organisms they are fed to (Prado et al., 2017, 2019a, 2019b).

In the case of bivalve mollusc hatcheries, the entire diet is based on phytoplankton, which is supplied to broodstock, larval cultures and seed. It is therefore a transversal compartment of the installation and influences all stages of the bivalves cultured.

This report presents the results of monitoring the presence of presumptive vibrios in the phytoplankton cultured in a batch system in a bivalve hatchery, to assess the risk of potential pathogen contribution by the feed pathway.

Materials and methods

The batch culture (BCS) is developed in the greenhouse of the Centro de Cultivos Mariños-CIMA (Ribadeo. Xunta de Galicia), in transparent polyethylene bags with a capacity of 40 l. The seawater used is filtered down to 1μm. The nutrient medium is Cell-Hi F2P Varicon Aqua, based on Guillard F/2 medium with trace elements, N, P and vitamins. The system has constant aeration and natural lighting, supplemented with LEDs to achieve a day:night photoperiod of 18:6. The microalgae grown are *Isochrysis*, *Diacronema*, *Tetraselmis*, *Phaeodactylum*, and occasionally benthic species. At times of peak demand, the required volume can be supplemented with a natural bloom obtained in the same facility.

Routine microbiological monitoring revealed the regular presence of suspected vibrios in the BCS mixture, to a greater or lesser extent, throughout the year. To verify this and identify the vibrios present, a specific control protocol was designed, sampling the mixing tank weekly, but also the monospecific ones. Temperature and pH data were collected for each sample to evaluate the possible influence of these parameters.

The samples were immediately processed on site, spread on plates of Thiosulphate-Citrate-Bilis-Bilis-Sucrose (TCBS), selective for vibrios, following Prado et al. (2014).

Results and discussion

Sampling was carried out during the year 2020, although unfortunately confinement and restrictions due to COVID-19 prevented us from obtaining the complete series.
In the first stage, January-February, *Vibrio splendidus*-like (VSL) was recurrently isolated. VSL bacteria have been linked to episodes of larval mortalities in hatcheries, although with an as yet unidentified pattern, different from the usual pathogens. The occasional detection of *Vibrio tapetis*, a known pathogen of adults but not of larvae, should also be noted.

At the beginning of the second stage, after containment by COVID-19, the pathogen *Vibrio alginolyticus* was already present. This species had been detected as predominant at times of high temperatures in the course of previous work. Initially, it was accompanied by VSL. During the summer, *V. alginolyticus* (/*V. parahaemolyticus*) persisted. Occasionally *V. mediterranei* and *V. harveyi* were detected.

In the September samples *Vibrio europaeus* was detected together with *V. alginolyticus*. It is likely that this pathogen was present previously, but overgrowth of *V. alginolyticus* at high temperatures may mask other species. Since its emergence, *V. europaeus* persisted together with *V. alginolyticus* until October. During September-October, the presence of *Vibrio neptunius* and, occasionally, the crustacean pathogen *Vibrio penaeicida* was also observed.

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Of the monospecific cultures, *Tetraselmis* and *Phaeodactylum* had the highest vibrio load, quantitatively and qualitatively, with a predominance of *V. alginolyticus*. The bacterial load in the cultures of *Isochrysis* and *Diacronema* with growth was generally low, although it should be noted that in addition to *V. alginolyticus*, *V. europaeus* was detected in these cultures.

A separate mention should be made of the occasional samples of natural bloom (August-October.) In all cases, the load was high or very high, and with a great diversity of potentially pathogenic vibrios for marine aquaculture, despite the small number of samples: *V. alginolyticus*, *V. neptunius*, *V. penaeicida* and *V. mediterranei*.

In summary:

- Monitoring shows that phytoplankton cultures carry vibrios, including potential marine aquaculture pathogens, which needs to be taken into account in the management of the facilities.
- In general, VSL is associated with the winter months (temperatures < 20ºC), while *V. alginolyticus* has a very important presence in the summer months (temperatures > 20ºC). *V. europaeus* was also detected.
- The pathogens described do not affect the microalgae, which act as "asymptomatic carriers".
- The load of vibrios in the phytoplankton is highly variable during the year, but also over the course of a week, probably related to factors such as cleaning protocols.
- It was observed that it was not uncommon to find a higher load in the distribution tube than in the mixing tank from which it started, indicating a possible point of bacterial persistence, probably related to the ability of many pathogens to form biofilms (Prado et al., 2019a).

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Acknowledgements

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Vibrio pectenicida IN CLAM CULTURES IN HATCHERY

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Introduction

The Centro de Cultivos Mariños-CIMA (Ribadeo. Xunta de Galicia) specialises in the development of bivalve molluscs from spawning to the appropriate size for outdoor cultivation. They have been diversifying the bivalve species, so that the facility is active throughout the year, combining clam and solenid cultures, taking into account the most appropriate season for each of them.

The USC-CIMA group has been developing microbiological control protocols for the facility, which include the transversal compartments (seawater circuit and microalgae cultures), as well as the larval, post-larval and spat cultures. Controls of larval cultures are of particular relevance, and are mainly aimed at detecting possible pathogens that may condition their development.

In this scenario, an outbreak of a pathogen that has not been detected so far in the hatchery, in the past years of microbiological controls, has arisen and seems to affect all the species cultivated at the time of its appearance, and to persist in the system.

Materials and methods

The known aetiological agents of hatchery mortalities are almost all bacteria of the genus *Vibrio*. Considering this, and that sampling in a hatchery must be a simple and quick process that does not interfere with the work of the personnel, the Thiosulphate-Citrate-Bilis-Sucrose (TCBS) medium, selective for vibrios, was used in the samples taken in routinary controls. Recently, it has been decided to incorporate the general medium Marine Agar (MA) as a standard, in order to broaden the spectrum of bacterial detection. Samples were taken and immediately spread on plates in the hatchery and further processing was carried out by the USC-team. Bacterial isolation, preservation and identification of isolates followed the methodologies described in Prado et al. (2014).

Results and discussion

Results showed the occurrence of *Vibrio pectenicida* (Lambert et al., 1998; Nicolas et al., 1996) in samples from the spawning of the growing carpet shell clam in May. Since then, this pathogen has been detected in all the developing cultures during June-July, both carpet shell clam, *Ruditapes decussatus*, and wedge shell clam, *Donax trunculus*, as demonstrated by 16S rDNA sequences obtained for the isolated strains.

The extent to which the cultures have been affected is being assessed, but there were problems in any case. To our knowledge, this is the first detection of *V. pectenicida* in hatchery cultures of carpet shell clam and wedge shell clam.

The addition of the MA medium to the microbiological controls has allowed the detection of a pathogen that does not grow in TCBS, and is therefore outside the routine controls in this bacteriological medium.

Since its initial detection, its presence has been recurrent, regardless of the bivalve species and the different spawns obtained. This fact seems to point to the facility in some way as the origin. It is not likely that different batches of broodstock of different species would carry the same pathogen simultaneously. Hypotheses point to an entry of the pathogen via seawater or phytoplankton and perhaps subsequent persistence through mechanisms such as surface biofilm formation. In other works, we demonstrated the recurrent presence of vibrios in the phytoplankton, as well as the ability of aquaculture pathogens within the genus to survive in this specific environment (Prado et al., 2019a, 2019c). We have also experimentally demonstrated the ability of many pathogenic vibrios to form biofilms on surfaces under hatchery conditions (Prado et al., 2019b), which makes them highly resistant to many of the treatments commonly used in these facilities.

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Acknowledgements

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BACTERIOLOGICAL STUDY OF A MORTALITY EPISODE OF EUROPEAN CLAWED LOBSTER, *Homarus gammarus*, IN EXPERIMENTAL CULTURE

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Introduction

All aquatic crustaceans are susceptible to Vibrio infection and the diseases associated with these pathogens may cause substantial production losses (de Souza and Wan, 2021). However, within crustaceans, some groups have been more deeply studies, while about others the literature is scarce. This is the case of the European clawed lobster, *Homarus gammarus*, clearly less well understood than their American counterpart, *H. americanus*.

Within the framework of the educational activities for aquaculture technicians of the IGaFA (Instituto Galego de Formación en Acuicultura), a lobster culture was developed using two different techniques. The sudden mortality of a batch led to microbiological sampling, especially aimed at identifying the vibrios presente, in order to gain a first insight into the microbiota associated with this species, with was little known until now.

Materials and methods

This report describes the microbiological sampling of a *Homarus gammarus* culture carried out at IGaFA's facilities in October 2020 using two different techniques. The classic method uses open flow, high water renewal and frozen food (Artemia). The alternative method is in stagnation with seawater renewal every two days, live food (Artemia) and phytoplankton (*Isochrysis galbana*). Both use seawater filtered to 1μm and sterilized by UV (Richards and Wickins, 1979, Uglem et al., 2006; Browne et al., 2009).

A massive mortality was observed in the alternative culture, close to the moult from stage II to III, not directly attributable to culture conditions. Samples were then collected for microbiological analysis from the alternative but also the traditional culture, as both were made with larvae from the same batch.

Samples were immediately processed in situ: larvae, gills, culture seawater, Artemia and *I. galbana*. Bacteriological media Marine Agar (MA) and Thiosulphate-Citrate-Bile-Sucrose (TCBS) were used. Bacterial isolation, preservation and identification of isolates followed the methodologies described in Prado et al. (2014).

Results and discussion

The bacterial load (TCBS, presumptive vibrios) of L4 larvae (alternative technique) was much higher than that of K1 (traditional system) larvae in larvae, gill and culture water samples.

In the TCBS samples of L4 larvae, *Vibrio rotiferianus-campbellii* and *Vibrio alginolyticus* grew as the major colony types. *Vibrio chagasii*, *Shewanella* and *V. rotiferianus-campbellii* were detected in gills. *Vibrio rotiferianus-campbellii* is also present in the culture seawater. In all cases, the same type of the Splendidus clade appears too.

In the K1 larval samples the bacterial load was lower and the diversity higher, sharing with the L4 larvae the presence of *V. chagasii*. It is interesting the presence of two potential pathogens, *Vibrio penaeicida* and *Vibrio alginolyticus,* both in the larvae and in the gill samples.

Looking for the origin of the vibrios, UV-treated seawater, microalgae and open-circuit seawater can be ruled out, with no growth in TCBS medium.

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As for the Artemia samples, the bacterial load (TCBS) of Artemia+cysts was too high, even in the 1 μl seeding, to differentiate specific colonies. In the sieved Artemia samples, *V. rotiferianus-campbellii* was isolated as a clearly dominant type, which points to this being the origin. *V. alginolyticus* was also detected, although in low concentrations. There was no growth in TCBS medium from the open-circuit water sample.

This is a first approach to the knowledge of the vibrios (and other bacteria) in hatchery cultures of *H. gammarus*, with the report of the presence of known aquaculture pathogens associated to mortalities, as well as a first attempt to elucidate the route of entry.

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DIFFERENTIAL PHOSPHORUS UPTAKE BY JUVENILE EUROPEAN CATFISH (*Silurus glanis*) FROM FEED AND WATER IN RECIRCULATING AQUACULTURE SYSTEM

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Introduction

In response to population growth and its effects on the environment and natural resources, there is a global need to exploit nutrient sources. Aquaculture is the fastest growing food production sector and accounts for 50% of the produced fish used for food (FAO, 2020). European catfish, *Silurus glanis*, has been farmed in Central and Eastern Europe for over 100 years. Absence of scales and of intramuscular bones and its favourable taste makes it an important species in the European aquaculture industry (Linhart, O et al., 2002).

Phosphorous (P) is an essential element involved in several life processes of fish such as energy transport and bone mineralization (Lall et al., 1979) In modern fish feeds, P is a limiting nutrient and supplementary mineral phosphates have to be added to the diet. The excretion of indigestible P leads to an accumulation of P in closed water systems, like recirculation aquaculture systems (RAS). Previous studies show that freshwater tilapia species can absorb P from the water through the gills and gastrointestinal tract (Al-Kholy et al., 1970; Urasa et al., 1987) and in other species, it has even been shown that the accumulation of this mineral in water can be beneficial for growth and normal skeletal development (Strauch et al., 2019; Van Bussel, C et al., 2013).

The objective of this study is to quantify the impact of water-borne P and dietary P at different concentrations on P-retention in juvenile European catfish. The final goal is to find an equivalence ratio to reduce the dietary phosphorus content by recycling the P accumulated in water. Differences will be assessed by quantifying fish growth, P-accumulation in the whole body and opercula and comparing the skeletal development and the total fat content.

Materials and methods

Juvenile *Silurus glanis* with an average starting weight of 1.79±0.17g were divided equally among 12 tanks of four independent RAS. The four RAS were set to contain four different freshwater phosphorous concentrations using mono-sodium phosphate (MSP): <10mg P/L, 40mg P/L, 80mg P/L, 120 mg P/L. In each system four different diets were tested with supplemental MSP: a control diet with 0.5% of total P (D1) and 3 increasing levels of dietary total P: 0.55%P (D2), 0.60% P (D3), 0.65% P (D4). Each diet was tested with three replicates per system at 23.4±0.7ºC in freshwater. Growth performance of each treatment was assessed after 21-days and 42 days. At trial end, fish were sampled and analysed for P in opercula, and whole-body P. Samples were also taken for skeletal development analysis by x-ray images (Fig.1).

Results (after 21 days)

Weight (g) of fish in different feeding and water treatments showed no significant difference after a 21-day growth period at either water P levels (P0, P40, P80, P120), or dietary P levels (D1, D2, D3, D4) (Fig. 2).

Discussion

At the end of the trial, data will be collected on whole body-P and operculum-P, fat content, skeletal development and final weight. An equivalency factor will be calculated which allows to reduce dietary P supplementation in relation to any given concentration of water-borne P.

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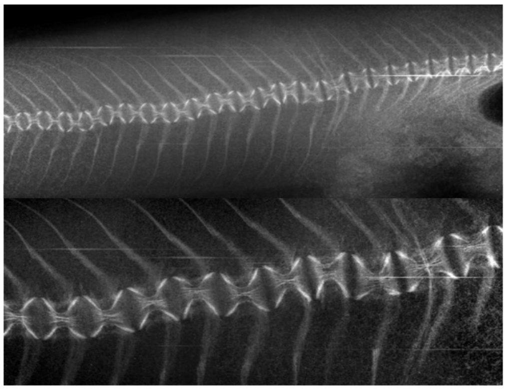

Fig.1. X-ray images test of juvenile European catfish at 10µm and 20µm scan (example micrograph for method development).

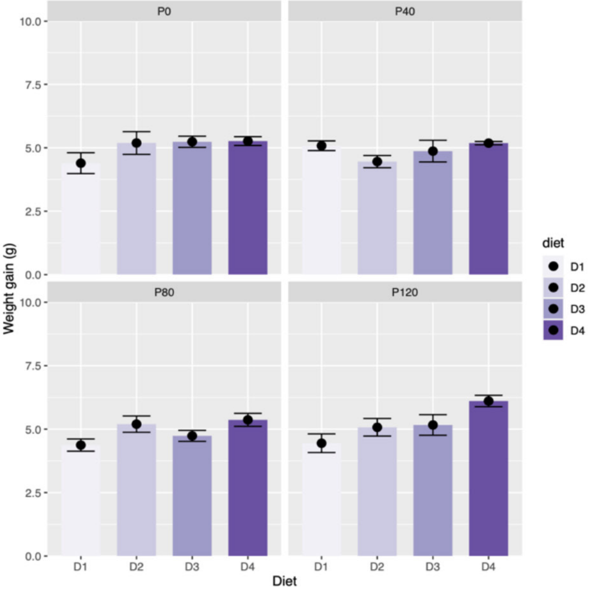

Fig. 2. European catfish weight gain (g) after 21-day growth period in the different water-borne P concentrations fed with increasing low dietary P levels.

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HERITABILITY ESTIMATES AND GENOMIC PREDICTION OF KOI HERPESVIRUS DISEASE (KHVD) USING A SUBSET OF SNP MARKERS ON TWO AMUR MIRROR CARP POPULATIONS

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Introduction

Koi herpesvirus disease (KHVD) can cause severe mortality in farmed carp populations causing major economic losses and is listed as a notifiable disease by the World Organization for Animal Health (Taylor et al., 2010; OIE, 2018). In the past years, the genetic architecture of resistance to KHVD has been widely studied (**Ødegård** et al., 2010; Tadmor-Levi et al. 2017; Palaiokostas et al., 2018, 2019; Zhao et al., 2020). Genome-wide SNP markers generated by RAD sequencing revealed significant QTL affecting resistance to KHVD (Palaiokostas et al., 2018) and led to optimizing genomic prediction of host resistance to KHVD in Amur mirror carp - AMC (Palaiokostas et al., 2019). The aim of this study was to assess the heritability estimates and the genomic prediction accuracy of such resistance using only a subset of SNP markers selected based on the previous genetic association to KHVD resistance (Palaiokostas et al., 2018; 2019) in two AMC populations.

Materials and methods

Two populations of AMC were created using a partial factorial design of 20 dams and 40 sires (Pop 1) and 27 dams and 29 sires (Pop 2). One-year fish (Pop 1 = 1,259 ind.; Pop2 = 1,325 ind.) were pit-tagged, phenotyped, fin clipped, and challenged to KHVD resistance as described in Palaiokostas et al. 2018. Restriction-site associated DNA sequencing (RAD-seq) generated 15,615 genome-wide SNP markers (RAD) on Pop 1 (Palaiokostas et al., 2019) from which 216 SNPs (LAB) with the strongest association to KHVD resistance were selected and genotyped on Pop 2. Genetic parameters and estimated pedigree (EBV) and genomic (GEBV_RAD and GEBV_LAB) breeding values were calculated and evaluated on Pop 1 using either pedigree, RAD or LAB markers and on Pop 2 using pedigree and only LAB markers. The heritability estimates and breeding values were estimated using the software package BLUPf90 (Misztal et al., 2014) and rrBLUP (Endelman, 2011). Moreover, 50 replicates of Monte Carlo 'leave-one-group-out' cross validation test was run to evaluate the accuracy of (G)EBVs on Pop 2.

Results

The percentage of total mortality was 66% for Pop 1 and 56% for Pop 2. After quality control (QC) of LAB markers, 183 SNPs (Pop 1) and 165 SNPs (Pop 2) were used for further statistical analysis. The heritability of KHVD resistance (Pop 1) as measured by survival on the underlying scale was estimated to be 0.61 using pedigree and 0.50 (RAD) and 0.36 (LAB) using genomic markers. On Pop 2, heritability estimates were 0.96±**0.09** (pedigree) and 0.68±**0.09** (LAB), and 0.78±0.25 (pedigree) and 0.55±**0.16** (LAB) with a model containing a random maternal effect. The phenotypic correlations (Pop 1) between EBV vs. GEBV_RAD, EBV vs. GEBV_LAB and GEBV_RAD vs. GEBV_LAB were very high: 0.91, 0.81 and 0.86, respectively. The phenotypic association between EBV vs. GEBV_LAB on Pop 2 was 0.79 and on average, GBLUP accuracy (0.66) was 7% higher than pedigree-based BLUP accuracy (0.62).

Discussion and conclusion

Heritability estimates were very high regardless of the challenge-tested populations of AMC and regardless of using the pedigree or the genomic relationship matrix. It repeatedly suggests that resistance to KHVD is a highly perspective trait to be genetically boosted by a selection program (**Ødegård** et al., 2010; Tadmor-Levi et al., 2017; Palaiokostas et al., 2018; Zhao et al., 2020). On the other hand, genomic heritability (Pop 1) using reduced LAB markers decreased (0.36) in comparison to the full density RAD markers (0.50). Still, it suggests that the selected subset of SNP markers captured a significant part of genetic variation for resistance to KHVD. Furthermore, the implementation of a subset of LAB markers into a genomic selection would allow a gain in accuracy of 7% compared to PBLUP selection on Pop 2, and with only

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a minor loss of accuracy when compared to GEBV_RAD (Palaiokostas et al., 2019) using a full SNP panel on Pop 1. It may be concluded that selected subset of SNP markers previously associated with KHVD resistance might be used for more accurate prediction of breeding values for KHVD resistance in different populations of AMC. Hence, it might be an interesting strategy to achieve the optimal balance between economic cost (number of SNPs to be genotyped per fish) and selection accuracy, as also recently investigated by e.g., Kriaridou et al. 2020 and Griot et al. 2021.

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EFFECT OF PEPTIDE DIETS ON HAEMAL LORDOSIS INDUCTION IN JUVENILE ZEBRAFISH (*Danio rerio*)

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Introduction

Peptides are considered advantageous for larvae improving physical properties (Piggot et al. 1982) and nutritional value of the diet (Carvalho *et al*. 1995). Reported to have a beneficial effect in fish performance (growth, survival) and skeletal development in several species (Szlaminka *et al*. 1993, Cahu and Zambonino 1995, Zambonino *et al*. 1997), they allow effective and early substitution of live with inert diets, reducing production cost. Raised hypotheses suggest protein hydrolysates' potential to enhance better absorption of the protein intake or fulfillment of amino acid requirements during the early larval stages (Cahu *et al*. 2003). Recently, a correlation of hydrolysate incorporation in fishmeal and regulation of skeletogenesis procedures has been described (Gisbert *et al*. 2012). Simultaneously, myogenesis can be affected by similar diets (Canada *et al*. 2018), in terms of muscle growth, degree of muscle fibers recruitment and hypertrophy activation.

Given the clear effect of peptides on musculoskeletal structure and activity in fish, the goal of the present study is to answer whether and how dietary peptides can prevent or reduce the occurrence of lordosis in a model fish species undergoing a swimming challenge at early juvenile developmental stage.

Materials and methods

Two experimental (P6, P12) and one control (C) diets were tested in triplicate, from the stage of yolk-resorption up to early juveniles in zebrafish (Fig. 1). Two feeding regimes were followed, with the incorporation (A) or not (B) of a short live feed period at the beginning. To evaluate bones' integrity against haemal lordosis induction, early juveniles with normal skeletal development were subjected to swimming challenge test (SCT) according to Printzi *et al*. (2021). Samples were collected immediately after the end of the SCT. Study of juvenile quality and the outlining of bone changes were succeeded through means of whole mount staining, histology and gene expression analysis of selected targets.

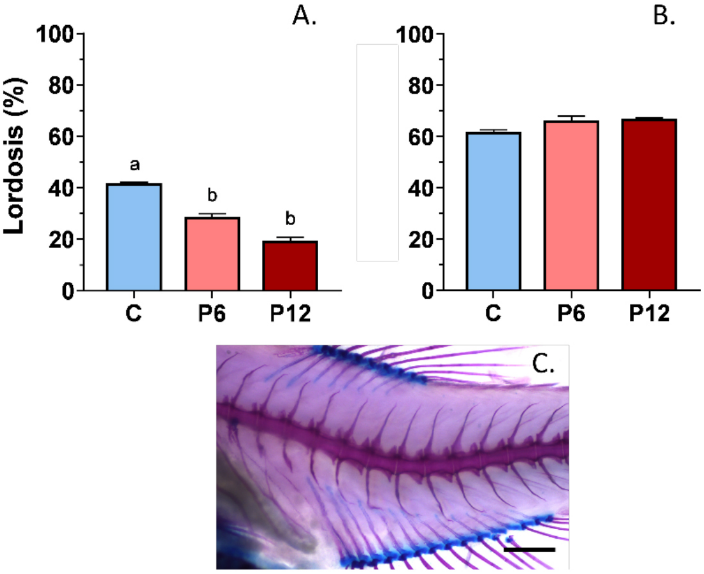

Figure 1. Lordosis frequency after SCT among the experimental diets on zebrafish juveniles, reared with (B) or without (A) live food incorporation. Lack of common letter indicates statistically significant differences.

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Results and Discussion

Peptide diets can alter the frequency of exercise-induced lordosis (Fig. 1C) observed after the end of the SCT regardless of the feeding regime. Exclusive supply of the dry diets (Fig. 1A) led to a decreased frequency of lordosis ranging from 41.9 ± 0.2 at group C to 28.6 ± 1.3 and 19.4 ± 1.2 at P6 and P12 respectively. In contrast, incorporation of live feed resulted in a common increased presence (61.8, 66.4 and 67.1%) of lordotic incidents between the diets (Fig.1B).

The correlation of harmonious axial development with incorporation of small peptides in fish diets seems to be coincident with the up to now findings of the present study. Understanding potential mechanisms through which peptides affect the musculoskeletal system is raised as a target. Direct beneficial effect for aquaculture will be integration of peptides in larval diets to maintain the optimum product quality. In addition, a possibility of skeletal deformities recovery (Fragkoulis et al. 2019) through proper nutritional handling will arise on a vertebral model species.

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A PREDICTIVE MODEL FOR THE ACCUMULATION OF OKADAIC ACID IN *Mytilus galloprovincialis* IN THE NORTHERN ADRIATIC SEA

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Introduction

Over the last decades, harmful algal blooms (HABs) have increased in frequency, duration, and severity worldwide, in association with storm events, changes in upwelling dynamics, climate change, and anthropogenic activity (i.e., animal waste inputs, agricultural runoff, growing aquaculture industry in coastal areas) (Grattan et al., 2016; Hallegraeff, 2010). Bivalve molluscs, by ingesting large amounts of phytoplankton, during HABs could become unsafe for human consumption. Dinoflagellates blooms of the genus *Dinophysis*, which produce okadaic acid (OA) causing Diarrhetic Shellfish Poisoning (DSP), are endemic to the Mediterranean coasts of Europe. To protect the consumers, European Union established mandatory monitoring of shellfish production areas on a weekly basis (EU Commission, 2019). Whenever the OA concentration in shellfish exceeds the established legal limit (equal to 160 µg kg-1 of OA equivalents, EU Council, 2004), shellfish harvesting activities are compulsorily suspended. The mussel farming areas along the Italian coasts of the Northern Adriatic Sea are affected by recurrent DSP events, in particular the Gulf of Trieste (France et al., 2006) and the marine area facing the Po River Delta (Veneto, Italy) (Pistocchi et al., 2012). In these areas, the main detected toxic species are *D. acuminata*/*D. sacculus* in spring-early summer, and *D. fortii*/*D. caudata* in late summer/autumn, associated with a predominance of OA in mussels. The impact of *Dinophysis'* blooms is expected to increase in the future, together with the expansion of mariculture. Mitigation of such phenomenon should prioritize research on the genetic regulation of toxin uptake pathways, absorption, and elimination mechanisms, and on predictive models for OA accumulation in bivalve molluscs.

This study aimed at developing a machine learning predictive model for OA accumulation in farmed Mediterranean mussel (*Mytilus galloprovincialis*) in the coastal area off Po River Delta (Veneto, Italy), based on oceanographic data measured by means of remote sensing. The results of the predictive model will be integrated into an online early warning tool available to shellfish farmers within the framework of AQUACULTURE2020 and VALUESHELL projects, funded by the Italian Ministry of Agriculture (MIPAAF).

Materials and methods

The data deriving from the official monitoring activities carried out from 2016 to 2020 to verify the accumulation of OA in the shellfish production sites located in the marine areas overlooking the Po River Delta were evaluated and analyzed. In this study, offshore sampling stations were considered those with the highest satellite coverage. The following physical and chemical parameters relative to the 6-weeks before each mussel sample (weekly mean, minimum, maximum, variance, skewness, and kurtosis) were associated with each OA essay: chlorophyll concentration (Chl, mg m^{-3}), Dinophyta contribution to total [Chl] (mg m^{-3}), eastern and northern components of wind direction (m s^{-1}), thickness of water column mixed layer (m), NH_4 concentration ([NH_4], mmol m^{-3}), NO_3 concentration ([NO_3], mmol m^{-3}), dissolved O_2 concentration ([O_2], mmol m^{-3}), PO_4 concentration ([PO_4], mmol m^{-3}), nitrates/phosphates ratio, daily precipitations (mm day^{-1}), salinity (psu), surface water temperature (°C), eastern and northern components of sea current (m s^{-1}), wind pressure on sea surface (Pa), solar radiation (Wh m^{-2}).

Data were classified according to a binary system: "1" for samples with a toxin value above the limit of quantification of the analytical method (LOQ = 40 µg kg^{-1}), "0" for samples with a toxin value below the LOQ. Data were divided into 1) train set, consisting of 1057 samples, collected before 2019 (of which 155 in class "1"); 2) test set, consisting of 429 samples, collected after 2019 (of which 89 in class "1"). Given the unbalanced dataset (dominance of the class "0"), the "1" category was oversampled using SMOTE (Synthetic Minority Oversampling Technique, Chawla, 2002). Different machine learning algorithms were evaluated with a cross-validation procedure, leading to define the LightGBM algorithm as the most suitable (Ke, 2017).

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Results and Discussion

The results of the classification algorithm on the test set showed an accuracy of 85%. Further analyses showed that false negatives were mainly associated with relatively low levels of toxins (< 100 µg kg^{-1}), while true positives had higher mean values of toxins: the algorithm tended to classify low concentrations of OA as negative samples. Since exact toxin concentrations for negative samples were not available (the LOQ of the analytical method was 40 µg kg^{-1}), it was not possible to analyze trends in false positives (e.g. concentrations just below the LOQ value, classified as positive). However, they tended to occur in August.

Further steps to improve the performance of the model will be: 1) to increase the number of available data, mainly of positive samples (class 1); 2) to correlate data on OA accumulation in mussels with data on *Dinophysis* spp. concentrations in the water column; 3) to include data on mussel physiology (e.g., dietary preferences depending on the structure of the phytoplankton community) in the model.

Once optimized, the predictive model will be integrated into the AQUAX online platform (https://gis.aquaexploration.com) as an early warning system capable of alerting mussel farmers 2/3 days before a highly probable OA accumulation event.

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EFFECT OF DIFFERENT STUNNING METHODS ON THE SHAPE OF *Salmo carpio* (L. 1758) FILLETS DURING *Rigor mortis* AND ICE STORAGE

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Introduction

There is growing interest among producers and consumers in ethical practices that safeguard fish welfare. Poor management during the stunning/slaughter phase can represent a stress condition, greatly compromising fish fillet quality during storage [1,2]. This has led aquaculture to adopt a comprehensive approach to ensure humane stunning and slaughtering practices and preserve fillet quality [3,4]. Slaughter-related stress generates H^+ ions associated with a high production of lactic acid. Consequently, pH diminishes in the early *post-mortem* stage, affecting fillet quality, *e.g.*, water holding capacity and texture [5-7]. Fish response to slaughter-related stress is species-specific. Thus, one of the most ambitious challenges is to find the best-performing stunning/slaughtering procedure for each species. Further studies are needed, especially for species poorly studied but with a high market value. This study was focused on "carpione del Garda" (*Salmo carpio* L.), an endemic salmonid of Lake Garda (Italy), which has been inserted as "critically endangered" into the Red List of Threatened Species [8]. Local farmers made several attempts to rear this species, whose fillets have a high market value (35-40 €/kg), but *S. carpio* is still considered a not domesticated species, and the farming protocols follow those of rainbow trout, including stunning/slaughtering procedures. To increase useful knowledge on *S. carpio*, this study aimed at evaluating the effects of two stunning methods commonly utilized for salmonids (i.e., electrical stunning and immersion in iced water) on fillets' shape, during *rigor mortis* in refrigerated storage. An automatic image analysis protocol for the evaluation of fillet shape changes was developed to that end.

Materials and methods

The experiment was carried out at the Experimental Fish Center of Fondazione Edmund Mach (San Michele all'Adige, IT) (Min. Aut. n. 22/2019-UT) and the protocol was approved by Ethics Committee. A total of 81 individuals (347.4±68.9 g) were randomly sorted into three experimental groups of 27 fish each (9 fish per replica). The stunning methods tested were: 1) a lethal dose of anesthetic (AN; 400 mg L^{-1} of MS-222), used as control; 2) electrical stunning in an EG100 Fishkill (ES; 24 V, 4 seconds; Scubla, UD, Italy); 3) cold shock by immersion in iced water (ICE; tank 240 L, -3°C). After stunning, all fish were killed by percussion by trained personnel. From each group, 6 fish (2 for replica) were allocated to *rigor index* (RI) evaluation until *rigor* resolution [9]. The right fillets of 9 fish for each group (n=27, 3 for replica) were photographed at different time points (0, 3, 6, 9, 12, 15, 21, 48, 72, 96, 168 and 192 h *post-mortem*). Fillet images were captured with a 12 M-pixels camera, in a lightbox and illuminated with a 6000 K lamp covered with a light diffusor. All the images (n=324) were calibrated using a 24-color patch Color Checker. Using "auto-threshold" and "analyze particles" functions of ImageJ [10], binary images were obtained, and the following geometric features were measured: area, perimeter, length, height, and circularity of the fillet [$(4\pi \text{ Area})/(\text{Perimeter})^2$]. RI data were analyzed using a Generalized Additive Model (GAM) [11], using treatments (AN, ES, ICE) and replicates (*i.e.*, tanks) as factors, and time and individuals as smoothers. Two-way PERMANOVA [11] was carried out to test for significant differences among treatments during the time. Multivariate analysis of variance (MANOVA) was used to test the differences in shape parameters means between groups at each time. Analyses were performed in R-4.2.1 environment.

Results and Discussion

RI evolution (onset, duration, and strength) was significantly different among treatments (t=3.51, p<0.01), but not among replicates (t=0.64, p=0.52), being faster in ES and ICE than in AN, and achieving the highest intensity after 21 and 28 h *post-mortem*, respectively in ES and ICE. Shape features changed accordingly to RI evolution, with a slight delay. Significant differences in shape geometric features were evident among stunning methods (F=5.82, p<0.001), but not in time (F=1.23, p<0.21), nor interaction between the two factors (F=0.75, p=0.999). The main shape changes were linked

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to the *rigor* onset and resolution, associated with a contraction phase followed by a relaxation one. During the contraction phase, in all groups, a reduction of the fillets' area (*i.e.*, shrinkage) was observed, while during the second phase fillets tended to recover their initial area in all treatments. In any case, fillets recovered their initial area with a more marked reduction in AN (-5.88%) and ICE (-5.70%). Perimeter experienced a reduction, not recovered in the relaxation phase, more evident and significant in ICE (-7.73%, $p<0.05$) and ES (-7.52%, $p<0.05$) than AN group. A fillet shortage was also observed, more evident in ICE (-9.89%), and lengths did not recover their initial values ($p<0.05$) in all the groups. Height and circularity had an opposite trend, both increasing during the contraction phase and decreasing in the relaxation one. In all groups, initial height was almost recovered at the end of the period considered, as variation was not significant between T0 and T192. On the opposite, initial circularity was recovered only in AN, while ICE (11.29%) and ES (13.11%) showed significantly different values between T0 and T192.

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SUSTAINABLE SPONGE FARMING TO OBTAIN BIOACTIVE COMPOUNDS: *Chondrosia reniformis* NARDO, 1847 AS AN INNOVATIVE SOURCE OF COLLAGEN

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Introduction

Being sessile filter feeders, sponges have developed a wide range of unique chemical defences to deter pathogens and fouling organisms, representing a great reservoir of chemodiversity (Di Cesare Mannelli et al., 2021). The interest of the scientific community for this group as a source of bioactive compounds have raised exponentially over the past decades, leading to a critical decline of sponge populations worldwide (Webster, 2007) due to its uncontrolled harvesting together with the more and more frequent disease outbreaks (Di Camillo & Cerrano, 2015).

Chondrosia reniformis is a peculiar sponge species containing a high abundance of collagen, a protein of great interest for the pharmaceutical and nutraceutical industries. Effective cultivation methodologies are under development in several international laboratories. The implementation of cost-effective marine culture plants of the species could provide the opportunity to develop local blue economies, while protecting *C. reniformis* wild stocks.

Material and methods

Experimental plants for the cultivation of *Chondrosia reniformis* were set at 20 m depth using old artificial reefs along the coast of Alassio (Ligurian Sea, NW Mediterranean). Each module consists of a concrete cube of side 2 m, and the modules are organised in groups of five blocks, forming a pyramidal system. In total, 80 fragments were transplanted on 16 horizontal faces of the blocks (Fig. 1).

Structure from Motion (SfM) photogrammetry allowed the creation of three-dimensional (3D) digital reconstructions from a series of overlapping images using Agisoft software. This technique was applied to estimate sponge volume, and to monitor regeneration of the sponge cuts for a period of 9 months. From volume data, annual increment of sponge biomass (expressed as grams of dry weight per year per initial volume of sponge) and collagen production (grams of collagen per year per initial volume of sponge) have been estimated, using the approaches applied by Morganti et al. (2019) and Pozzolini et al. (2018), respectively.

Results

From the 80 *C. chondrosia* fragments transplanted, 40 were used to estimate yearly changes. 92.3% of the 40 cuts successfully attached to the substrate and all of them showed an increment in volume of 120% ± 63.14% per annum. Which means an estimated production of 125 ± 90 gDW year^{-1} dm^{-3} in terms of biomass, and an estimated production of 37.3 ± 27 g collagen year^{-1} dm^{-3}, considering the average yield established by Pozzolini et al., 2018.

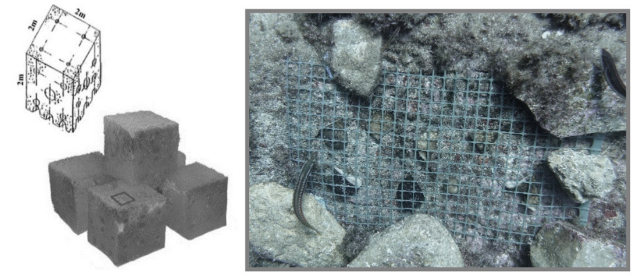

Fig. 1. Artificial structures used as substrate for mariculture implants of *Chondrosia reniformis*.

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Discussion and conclusion

The mariculture of *C. reniformis* has been repeatedly identified as a challenge in the literature, especially over horizontal substrates given its high motility (Gökalp et al., 2022). Our mariculture approach on artificial substrates highlighted once again the potential of this species as a source of marine collagen, by obtaining high re-attachment, survival and growth rates from the sponge explants.

The application for the first time of SfM-photogrammetry to monitor the growth of this species resulted in a complete success. Thanks to this technique, volume and surface fluctuations through time could be recorded along the experiment. The application of non-invasive 3D monitoring tools is therefore crucial to understand possible seasonal changes and growth patterns of this species over long-term series.

Our results showed that fragments of this sponge can double their volume in one year, suggesting the feasibility of the tested cultivation technique. Nonetheless, environmental data still need to be better analysed to evaluate the best local conditions to guarantee long-term investments in *C. reniformis* aquaculture.

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ALTERNATIVE PROTEINS OR ACTIVE INGREDIENTS? COMPARISON OF DIFFERENT DIETS ON *Dicentrarchus labrax* FILLET QUALITY AND OXIDATIVE STABILITY

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In recent years, aquaculture has proven to be the fastest growing sector in the agri-food industry. The use of non-conventional protein source responds to the need of the aquafeed sector to replace conventional dietary protein sources with eco-friendly and nutritious ones potentially acting also as functional ingredients. Considering the recent researches, *Hermetia illucens* meal (HM) and poultry by-product meal (PBM) can be considered valuable alternative protein sources fitting the ecological transition towards a circular bio-economy (Pulido-Rodriguez et al., 2021). Recently, dried marine microalgae biomass (MA) and red swamp crayfish meal (RCM) have also been seen as interesting supplements due to their valuable profile in n-3 polyunsaturated fatty acids (PUFA) and content in vitamins, minerals, and natural antioxidants (Zarantoniello et al., 2022). Due to this, the aim of the present trial was to evaluate the effects of five experimental diets contained the aforementioned protein sources on European sea bass fillet quality and oxidative stability during 60 days of frozen storage.

Material and methods

Two-hundred and seventy European sea bass (*Dicentrarchus labrax)* were equally and randomly distributed in 15 tanks (300 L) and maintained under controlled conditions during the whole experiment (T 23.6 ± 0.70 °C; salinity 30 ± 1.4 g/L; dissolved oxygen 6.1 ± 0.38 mg/L). Five grossly isoproteic (45%), isolipidic (20%) and isoenergetic (22 MJ/kg) diets, deprived of fish meal, were formulated starting from a vegetable protein-based feed and distributed to fish in 3 different tanks for each diet. The diets were prepared by partially replacing the plant proteins with MA (10%, 2:1 *Tisochrisis lutea* and *Tetraselmis suecica*; MA10), RC (10%, RC10), PM (20%, PM20) and HM (20 or 40%, HM20 and HM40, respectively). Fish were fed the experimental diets twice a day for 21 weeks. At the end of the trial, 8 fish per group were slaughtered by trained personnel and fully characterized during a storage trial. Immediately after death, the fish were filleted and the right-side fillets were analyzed at 0 day of storage (S0), while the left-side fillets were individually inserted into plastic bags and stored at -10 °C for 60 days (S60) in order to observe possible variation on lipid oxidation during the storage period at sub-zero temperature. Analyses of color (CIE, 1976)colour difference equations and metric colour terms","type":"article-journal"},"uris":["http://www.mendeley.com/documents/?uuid=56c606e1-e157-4327-a159-73e33dc10bc8"]}],"mendeley":{"formattedCitation":"(CIE, 1976, lipid content (Folch et al., 1957), fatty acid profile and lipid oxidation (Bruni et al., 2021) were assessed on the S0 and S60 fillets. A two-way ANOVA (main effects: diet, D; storage, S and their interaction, D×S) was applied for data analysis, followed by a Tuckey post-hoc test to compare the mean values considering a significant difference at $p<0.05$.

Results and discussion

Microalgae and red crayfish are rich in carotenoids which are responsible for flesh pigmentation (Pulcini et al., 2021). The analysis showed that the yellowness index (a^*) was increased by MA diet ($p<0.0001$), while the other diet did not affect any of the considered parameters, analogously to those found by Pulido-Rodriguez et al. (2021). The diets also modified both lipid content and the fatty acid profile of fillets. The HM40 fillets were fatter than fillets from MA10 and RC10 groups and they had the highest SFA content. The MA10 had the highest value of n-6PUFA and not different n-3PUFA level, but the n-3 fatty acids were singularly affected. For instance, HM20 and MA10 fillets were the richest in C18:3n-3 while HM40 in C20:5n-3. In this trial, it was found that the carotenoids present in the MA diet were able to preserve the fillets from lipid oxidation during the 60 days storage at -10 °C. In contrast, fillets from E. sea bass fed RC diet reached the highest value of lipid oxidation at the end of the storage. Hence, while the level of RC inclusion did not seem to preserve the quality characteristics of the fillets, the microalgae mixture could be taken into consideration in the case of fish intended for preservation or handling rather than for consumption as fresh.

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Color parameter values, lipid content, fatty acid composition and oxidative stability of E. sea bass, analyzed as fresh or after 60 days of storage at -10 °C.

	DIET, D					STORAGE, S		RMSE	D	S	D×S
	HM20	HM40	MA10	PM20	RC10	0	60				
*L**	52.02	53.33	52.35	51.56	51.46	49.96	54.32	1.50	ns	***	ns
*a**	0.01	-0.56	0.01	-0.33	0.14	0.63	-0.92	0.78	ns	***	ns
*b**	1.05^{b}	0.63^{b}	3.14^{a}	0.88^{b}	1.41^{b}	0.07	2.77	1.02	***	***	ns
Lipid content (g/100 g) and fatty acid profile (g/100 g FAME)											
Total lipids	7.05^{ab}	8.86^{a}	5.21^{b}	7.45^{ab}	6.55^{b}	6.71	7.33	2.46	*	ns	ns
C12:0	0.75^{b}	1.12^{a}	0.03^{c}	0.05^{c}	0.05^{c}	0.37	0.42	0.05	***	**	***
C14:0	2.28^{b}	2.53^{a}	1.86^{c}	1.76^{d}	1.83^{cd}	2.22	1.89	0.09	***	***	ns
C16:0	15.96	16.02	16.21	16.32	16.13	17.26	15.00	0.39	ns	***	ns
C16:1n-7	3.21	3.24	3.10	3.29	3.25	3.48	2.96	0.15	ns	***	ns
C18:0	3.40	3.42	3.28	3.59	3.54	3.38	3.51	0.27	ns	ns	ns
C18:1n-9	32.01^{b}	31.71^{b}	30.29^{c}	32.73^{a}	33.34^{a}	32.39	31.65	0.80	***	**	ns
C18:1n-7	2.33^{b}	2.42^{a}	2.40^{a}	2.41^{a}	2.39^{a}	2.42	2.36	0.04	**	***	*
C18:2n-6	14.25^{b}	14.29^{b}	17.12^{a}	14.50^{b}	14.52^{b}	15.07	14.81	0.42	***	*	ns
C18:3n-3	7.62^{a}	6.86^{d}	7.68^{a}	7.12^{c}	7.42^{b}	7.37	7.31	0.02	***	ns	ns
C20:5n-3	4.76^{b}	4.93^{a}	4.31^{c}	4.73^{b}	4.63^{b}	4.35	1.00	0.18	***	***	ns
C22:6n-3	2.71	2.77	2.99	2.69	2.56	5.42	0.07	0.53	ns	***	ns
Σ SFA	23.14^{b}	23.84^{a}	22.14^{c}	22.48^{c}	22.33^{c}	23.91	21.66	0.60	***	***	ns
Σ MUFA	40.13^{b}	39.98^{b}	38.23^{c}	40.88^{a}	41.46^{a}	40.62	39.65	0.96	***	**	ns
Σ n-6PUFA	15.44^{b}	15.53^{b}	18.48^{a}	15.79^{b}	15.69^{b}	16.07	16.30	0.44	***	ns	ns
Σ n-3PUFA	20.51	19.82	20.52	20.13	19.83	18.66	21.66	0.83	ns	***	ns
Lipid oxidation											
CD, mmolHp/100g	0.19^{ab}	0.23^{a}	0.15^{b}	0.20^{a}	0.18^{ab}	0.17	0.21	0.05	*	*	ns
TBARS, MDAeq.-/kg	0.17^{b}	0.16^{b}	0.13^{b}	0.17^{b}	0.26^{a}	0.03	0.32	0.07	**	***	**

HM, *Hermetia illucens* meal; MA, dried microbial biomass from *Tisochrisis lutea* and *Tetraselmis suecica;* PM, poultry by-products; RC, invasive crayfish *Procambarus clarkii* meal. The fatty acids C14:1n-5, isoC15:0, C15:0, isoC16:0, C16:1n-9, C16:2n4, C17:0, C16:3n4, C17:1, C16:4n1, C18:2n4, C18:3n-6, C18:3n4, C18:4n-3, C18:4n1, C20:0, C20:1n11, C20:1n7, C20:1n9, C20:2n-6, C20:3n-6, C20:3n-3, C20:4n-3, C22:0, C22:1n11, C22:1n-9, C22:1n7, C22:2n-6, C21:5n-3, C22:4n-6, C22:5n-6, C22:5n-3, C24:1n-9, found below 1%, are not reported in the table but they were utilized to calculate the Σ of each lipid class. FAME, total fatty acid methyl esters. CD, conjugated dienes; TBARS, thiobarbituric acid reactive substances. RMSE, Root Mean Square Error. * p-value <0.05; ** p-value <0.01; *** p-value <0.001; ns, not significant.

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FISH TRACKING FOR AUTOMATIC DETECTION OF REPRODUCTIVE BEHAVIOUR

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Introduction

Senegalese sole (Solea senegalensis) have good aquaculture potential. However, sustainable development has been hindered by a reproductive failure of cultured broodstocks, hatched and reared in captivity (Duncan et al., 2019). Cultured males exhibit a behavioural dysfunction and do not participate in courtship to fertilise eggs. A behaviour termed the "Follow", behaviour where sole follow each other in a procession and that is unique to the courtship has been associated to successful spawning. High participation in the "Follow" behaviour appears to indicate spawning success. The aim of the present study is to automate the identification of the "Follow" behaviour in videos of Senegalese sole broodstocks and to track individual sole involved in the behaviours.

Materials and Methods

The videos used for the behavioural analysis were taken from a broodstock tank that contained both wild and cultured breeders, formed with 2 wild males, 5 wild females, 5 cultured males and 4 cultured females (mean weight 1,192.61 ± 170.70 g). The broodstock had been held together during 4 years under natural photoperiod and simulated temperature conditions, fed daily and water quality was maintained optimal with a recirculation system (IRTAmar®). Video recording was during April at the peak of the reproduction season. The videos were recorded with cameras installed just below the water surface in the corner of the broodstock tanks and connected to a video recorder. The period recorded was during the night (19:00-20:00) with night illumination (Carazo et al., 2013) and when the locomotor activity of fish was highest, due to the reproductive behaviour. For the purpose of this study, five video clips of the fish participating in the "Follow" behaviour were randomly selected from 30 video clips of the "Follow" behaviours taken from days with spawning that had been previously identified and analysed. As control videos, a further five video clips were selected that were of the same group of fish and recorded during the same time period. These control video clips had fish swimming individually that were not participating in the "Follow" behaviour.

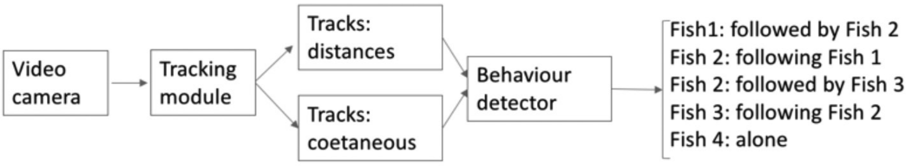

Figure 1. Main scheme of the tracking system.

Figure 2. Two shots from the fourth video. The video tracker has detected four fish.

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Results and Discussion

The main scheme (Fig. 1) of the tracking system included a tracking module that keeps the information of the tracks of all the fishes, which is updated with each frame. Tracks are composed of a vector that each element keeps the current position (x,y), the time and the fish identification. Note that the camera does not visualize the whole tank and for this reason, tracks have a beginning (when the fish appears in the visual field) and an ending (when the fish disappears from the visual field).

Ideally, the position has to be the centre of the fish. Note that this position is represented in 2D-camera coordinates. The track distances were computed using the algorithm called Dynamic Time Wrapping (DT) (Petitjean et al., 2011). This algorithm deduces a distance between vectors or discretised functions. Speaking in a plain language, the idea is to deduce the cost of transforming a vector into another considering that elements in the vectors have to keep the same order. It is based on three main edit operations, viz, substitution of local elements, deletion of elements in the first vector and insertion of elements in the second vector. In our case, we have only considered the 2D position of the tracks and the time has been discarded in this stage. Moreover, since tracks can have very different lengths, the final distance is normalised by the average length of the two involved tracks. The time information in the tracks is taken into consideration to deduce if tracks are coetaneous. We consider that two tracks are coetaneous if the beginning of both tracks are inside a specific temporal window TW.

The final output of the system depends on the track distances and also on the information if both tracks are coetaneous. We consider Fish 2 follows Fish 1 if they are coetaneous, the track of Fish 2 begins later than the track of Fish 1 and the distance between tracks is smaller than a threshold DT. On the contrary, we say that a Fish has an "alone" behaviour if the distance between it´s track and the rest of tracks that are coetaneous to it is larger than the threshold DT. Therefore, a DT lower $DT < 40$ indicated a fish was considered to have a "Follow" or "Follower" behaviour and contrarily, larger or equal to $DT \geq 40$ indicated an "alone" behaviour. Two shots from the fourth video in which there is a "Follow" behaviour, show that Fish 1 is followed by Fish 2 and Fish 3. Moreover, Fish 4 stays alone the whole video and does not relate to the other fish. Thus, we consider the four fish have different behaviours. Fish 1 is a "Followed", Fish 2 is a "Follower" and a "Followed", Fish 3 is a "Follower" and Fish 4 is an "Alone". The DT values for Fish 1, 2 and 3 in relation to each other ranged from 15.9 to 25.3, while distances between Fish 4 and Fish 1, 2 and 3 ranged from 85.6 to 97.2.

These promising results demonstrate a simple computer vision system to identify different behaviours related to courtship that can be used to detect and predict spawning behaviour in Senegalese sole. This system can be further developed to automatically detect the behaviours in real time without the need to make video recordings.

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ESTIMATION OF BIOFILM ACTIVITIES ON BIOFILTER CARRIERS FROM RECIRCULATING AQUACULTURE SYSTEM BY RESPIROMETRY

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Introduction

Biofilm, the aggregates of microbial layers that are attached on the surface of biofilter carriers, plays a central role in nutrients removal in recirculating aquaculture system (RAS) to achieve high degree water reuse for fish production (Chen et al., 2006). The bacterial activities in biofilm are of great importance for surveillance and management of RAS biofilter, but cannot easily be measured. Moreover, a biofilm detachment process and laboratory equipment are required for current methods to characterize the biofilm, which seem unsuitable for on-site application in a RAS facility. The present study was conducted with an aim to develop a simple and reproducible method that allows *ex-situ* estimation of biofilm activities without destroying biofilm integrity.

Materials and methods

Three different types of biocarriers, extruded polypropylene (EPP), injection molded polypropylene (IMPP) and polymeric foam (PF) from biofilters connected to the same RAS were tested in our tailor-made respirometry system. The oxygen consumption rates of biofilm in respirometric chambers packed with biocarries were measured after intermittent spiking with either ammonium, nitrite or acetate. The standard substrate degradation batch kinetics tests for evaluating biofilter performance were also conducted to confirm the feasibility of our proposed method as a means to assessing biofilter performance.

Results

Results showed that the method allowed estimation of endogenous respiration rates, as well as respiration of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and heterotrophic bacteria (HB) in biofilm. The highest activities, calculated and standardized as volumetric oxygen consumption rate (VOCR), were found in PF with values of 764, 677 and 166 g $O_2 \cdot m^{-3} \cdot d^{-1}$ for AOB, NOB and HB, respectively (**Figure 1**). Furthermore, substrate degradation batch tests matched respirometric tests well for all three tested carriers to evaluate biofilter performance (**Figure 2**).

Discussion and conclusion

The higher activities of AOB, NOB and HB in biofilm of PF carriers suggest that PF carriers could achieve more effective removal of ammonia, nitrite and organic matter in RAS water treatment. This is in support of findings from Shitu et al., (2020) that found PF carriers achieved higher nitrification performance than plastic media in MBBR treating RAS wastewater. The deviation between the predicted and observed values for biofilter performance for PF carriers may be the result of different factors, such as that i) respirometric tests and nitrification batch kinetic tests were performed separately, ii) differences in hydraulic conditions in reactors (closed respirometric chamber vs. open beaker) might have an influence on oxygen and substrate diffusion, iii) a part of ammonia, as well as small amount of electrons from TAN oxidation and nitrite oxidation processes are used for cell synthesis, which has a lower oxygen demand than predicted by the theoretical oxygen demand (Liu and Wang, 2012). In conclusion, the proposed method allows estimating activities of biofilm attached on biofilter carriers from a freshwater RAS by measuring oxygen consumption rates following substrate spikes. The designed intermittent respirometer platform achieved replicated and non-invasive oxygen measurement without disturbing biofilm integrity. This method allowed complete estimation of metabolic activity of endogenous respiration, NOB, AOB and HB in biofilm within less than five hours. Comparison study with standard substrate degradation kinetic batch tests for assessing biological processes in biofilter confirmed the feasibility of our proposed method to evaluate the biofilter performance. Our study provides a potential tool of monitoring and diagnosis for RAS biofilter and a better understanding of biofilm on the carriers, which would benefit biofilter design and water quality management in RAS.

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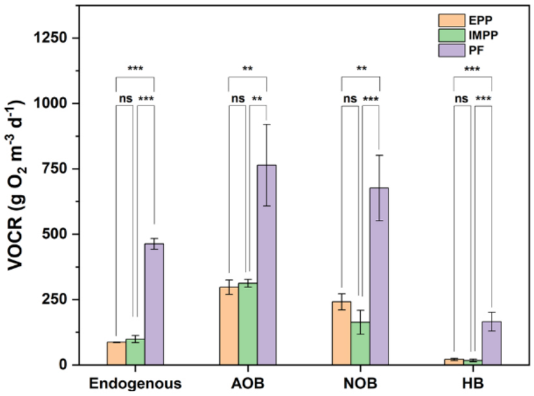

Figure 1. Volumetric oxygen consumption rates (VOCR, g $O_2 \cdot m^{-3} \cdot d^{-1}$) measured in three different types of carriers. Endogenous VOCR was measured in water without substrates, ammonium (AOB+NOB) and nitrite oxidation (NOB) related VOCR in presence of TAN or nitrite, respectively and heterotrophic activity (HB) during addition of acetate. EPP=Extruded polypropylene, IMPP=Injection molded polypropylene, PF=Polymeric foam. Bars and error bars denote mean ± standard deviation of the mean (n=3; $^{**}P<0.01$; $^{***}P<0.001$; ns=not significant).

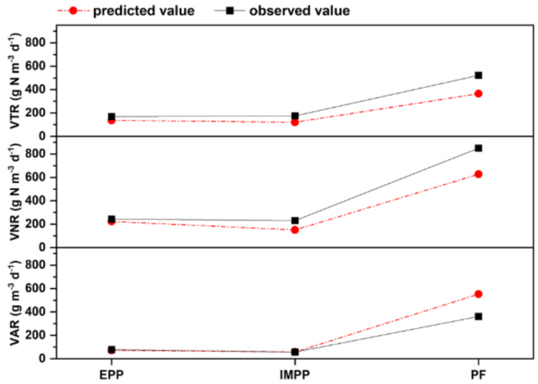

Figure 2. Biofilter performance assessment comparison between predicted values based on VOCR (volumetric oxygen consumption rate) data from respirometric tests and observed values from substrate degradation batch kinetic tests for three tested carriers (EPP=Extruded polypropylene, IMPP=Injection molded polypropylene, PF=Polymeric foam). VTR, VNR and VAR were volumetric TAN, nitrite and acetate removal rate representing as parameters for evaluating biofilter performance.

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DETECTION AND ELIMINATION OF MICROPLASTICS IN AQUACULTURE FARMED MACROALGAE

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Introduction

The REMEDIA Life Project (REmediation of Marine Environment and Development of Innovative Aquaculture: exploitation of edible/not edible biomass) funded by the European Community foresees, for the first time in Europe, the use of an integrated multi-trophic aquaculture system (IMTA) where a new set of bioremediator organisms, such as polychaetes, porifera, mussels and macroalgae, are reared/cultivated in addition to fish breeding. Macroalgae, in particular are included in the set of bioremediator organisms because some species are able to reduce the nitrogen and phosphorus load produced by fish farming. The project aims to demonstrate that the bioremediation technologies developed can be successfully applied to an industrial mariculture plant in an confined environment with positive effects in the specific sector and with the aim of the exploitation and the zero-kilometer marketing of the biomass produced. In particular, the biomass of macroalgae produced can be used for the extraction of active compounds or as food useful in the dietetic field. In order to verify the safety of macroalgae to be used as food the present work was aimed to verify the presence and potential removal of microplastics (MPs), which are among the most hazardous emerging pollutants (Sharma and Chatterjee, 2017), in *Chaetomorpha linum* (O.F Müller) Kützing (Chlorophyta, Cladophorales), one of the seaweeds collected in the Mar Piccolo of Taranto (Northern Ionian Sea, Mediterranean Sea) and then transferred to the aquaculture farm to be cultivated in the IMTA system.

Materials and Methods

Chaetomorpha linum, was collected during the season of maximum growth. Three replicates of about 500 g of fresh material were harvested by a rake at a depth of 50 cm. Before the placement of macroalgae in the plant, the presence and removal of MPs, from the thallus was evaluated by performing three extractions with a sodium chloride (NaCl) hypersaline solution (salinity of 38.00 ‰ and density of 1.20 g/cm^3) as reported by Thompson et al., (2004). Algae were placed in a beaker with a magnetic stirrer at 400 RPM for 20 minutes in the hypersaline solution and then let to settle for 2 hours. After that period the microplastics floated by virtue of their low density, the supernatant was filtered on a glass microfiber filter (GF/F, 47mm, 1μm) using a vacuum pump, then dried in the oven at 40 °C for 24 hours. Filters were inspected primarily using a stereomicroscope, each particle considered microplastic was photographed with a camera attached to the stereomicroscope. The particles were classified and catalogued on the basis of their shape and colour following the categories suggested by Hidalgo-Ruiz et al., (2012). To assess the chemical nature of the particles found, the samples were analyzed by ATR-FTIR spectroscopy. For this purpose, a Perkin Elmer Spectrum One Spectrometer was employed. The assignment of absorption bands in the output spectra was accomplished by comparison with spectra of standard polymer materials found in the literature (Jung et al., 2018). After the procedure of three times washing with NaCl solution algae were analyzed under a stereomicroscope in order to check the absence of MPs.

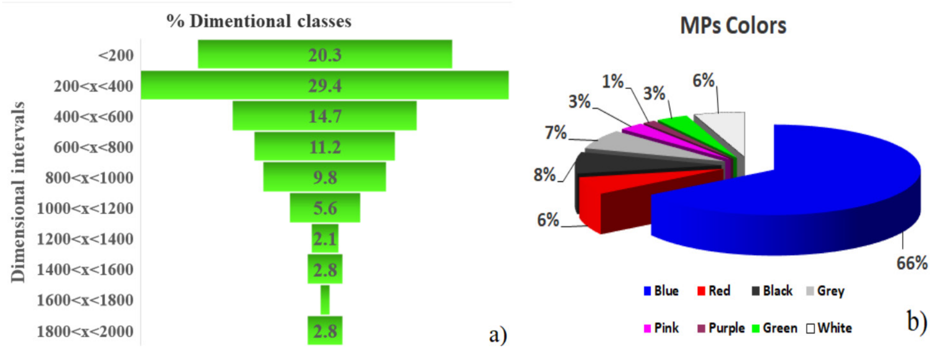

Fig 1 - Percentages of the different recorded MPs: a) dimensions; b) colours.

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Results and Discussion

Up to now information on the status of MPs accumulation in marine autotrophic organisms is scant even though they have been recently reported in macroalgae (Li et al., 2020). This is noteworthy taking into account that macroalgae serve as animal feed and human food (Gao et al., 2018). Our data suggest that in *C. linum* several kinds of MPs are present. Out of the total of 143 MPs found, 141 were fibers while only two were fragments. The average particle size was found to be 555 μm. The size class with a major number of MPs was between 200 μm and 400 μm (Fig. 1a). Among the various colours, the predominant was blue (65.7%), while the remaining colours had considerably lower percentages (Fig. 1b).

ATR-FT-IR spectra related to the relevant fibers were assigned to polypropylene, polypropylene plus calcium carbonate filler, polystyrene, polyethylene, and polyester reinforced with calcium carbonate (E.Quarta unpublished data). In conclusion, our findings highlighted the presence of MPs in *C. linum* and the possibility to remove them with simple procedures thus encouraging the exploitation of this species in an aquaculture scenario where it is cultivated in the realized IMTA system. Finally, the strong trapping capacity of MPs by *C. linum* indicates this seaweed as a potential material to remediate MPs-polluted seawaters

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VERTEBRAL ANOMALIES IN SENEGALESE SOLE (*Solea senegalensis*): MULTITECHNIQUE ASSESSMENT AND TRANSCRIPTOME PROFILE

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Introduction

Skeletal anomalies are rather frequent in Senegalese sole (*Solea senegalensis*) aquaculture. Particularly, vertebral deformities may impact physiological processes and welfare, affecting swimming, growth or even the susceptibility to other diseases, as well as slowing weight gain. Although several diagnostic techniques have been used for the assessment of skeletal deformities in Senegalese sole, the molecular mechanisms underlying the vertebral deformities are still poorly understood. In this context, the present multidisciplinary research aims at deepening the knowledge of the genetic basis and molecular pathways of osteogenesis processes and the development of vertebral anomalies in *S. senegalensis*.

Materials and methods

For this study, an experimental trial was carried out in the Marine Research Centre ECIMAT (Vigo, Spain). A commercial batch of Senegalese sole fertilized eggs was split into two groups subjected to two different incubation temperatures (18 ºC and 22 ºC). Hatched eggs were then placed in separate tanks, with three replicate tanks for each condition. Random samples of 300 postlarvae (30 days after hatching, dah) and 311 juveniles (130 dah) were taken to evaluate the prevalence of vertebral anomalies by stereomicroscopy. Stereomicroscopy, digital radiography, and digital imaging methods were performed to assess the vertebral anomalies at 30 and 130 dah, classify the main types of vertebral anomalies and support sampling collection for further transcriptomic analyses. Vertebral bone tissues from unaffected and anomalous vertebral phenotypes were collected for RNA extraction at 30 and 130 dah. Muscle and fin tissues were also sampled in the latter juvenile stage. An RNA-seq analysis including three replicates per condition was performed after quality-filtered reads aligned against the Senegalese sole genome. Reference mRNA and miRNA transcriptomes of vertebral bone were obtained for functional evaluation of bone homeostasis and spinal pathologies in the conditions tested.

Results and discussion

The data obtained from the stereomicroscopic study, focused on the count of vertebrae and the skeletal alterations present, showed significant differences between the incubation temperatures. At 22 ºC, both postlarvae and juvenile Senegalese sole presented a higher number of total vertebrae in the column, and the prevalence of vertebral anomalies was higher, especially the number of vertebral fusions. Regarding the transcriptomic analysis, sets of differentially expressed genes (DEGs) were detected between control and malformed vertebral phenotypes across tissues and life-stages under different incubation temperatures, providing insights into the functional mechanisms regulating vertebral development and growth in Senegalese sole. These results on DEGs revealed long-lasting effects of early incubation temperature on the spine, and regulatory insights on vertebral bone health and growth. Among these, defective extracellular matrix, osteogenesis, chondrogenesis, mineralization, bone remodelling and inflammation. Muscle and fin DEG profiles also correlated with cartilage and bone related genes associated with the spinal skeleton health.

Conclusion

This study shows the imprint of the environmental conditions of egg incubation on the bone tissue, which is manifested on the prevalence of skeletal malformations until juvenile stages. On the other hand, the transcriptomic study is useful for the identification of mRNA and miRNA biomarkers that may be applicable to aquaculture industry and to the comparative pathology of bone tissue.

Acknowledgements

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VERTEBRAL ANOMALIES IN COMMERCIAL SIZE CULTURED TURBOT (*Scophthalmus maximus*)

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Introduction

A high frequency of skeletal anomalies can represent a multilevel problem for the aquaculture sector, especially if they repercuss in the general appearance of the fish sold as a whole, causing rejection by consumers. Despite the high relevance of turbot (*Scophthalmus maximus*) in European aquaculture, there are few studies addressing vertebral anomalies at commercial size cultured fish. Reports at early stages of development, detected the presence of skeletal anomalies in around 50 % of the turbot larvae/post-larvae (Tong *et al*. 2012). Conversely, only 2.3 % of fish at 0-47 days after hatching (dah) showed spinal deformities in Lv *et al*., 2019. This work aimed to characterize the main vertebral body anomalies and spinal deviations on the last stage of turbot rearing, close to market size, by means of computed radiography. The relationship between deformities and biometric measurements of the fish was assessed.

Material and methods

For this study, 51 farmed turbot at commercial size were radiographed in latero-lateral (LL) projection using a Philips Super 80 CP X-ray equipment and a mammography film (FCR Fuji IP cassette type CH). Exposure parameters ranged from 40 kV to 45 kV and 25 mAs, depending on the fish size. Computed radiographic images were analysed with a RadiAnt DICOM Viewer 2020.2.3 software. External biometric measurements were taken as standard length (StL; cm) and maximum body width (BW; cm), and StL/BW ratio was calculated. Vertebral bodies were assessed for the presence of anomalies, according to Witten et al., 2009. To check the relationship among fish biometrics and deformities, individuals were distributed in three groups ("D1-5", "D6-10", "D>10") according to the number of deformed vertebrae. The first group "D1-5" included fish with 1 to 5 deformed vertebrae; the second group "D6-10" included fish with 6 to 10 deformed vertebrae; and fish with more than 10 deformed vertebrae were allocated in group "D>10". Group differences in StL, BW and StL/BW ratio were analysed using one-way ANOVA test. Normality and homogeneity of variance of the data were previously checked using Shapiro-Wilk and Levene's tests, respectively. Tuckey's pairwise comparison tests were performed in case of finding significant differences ($p < 0.05$).

Results and discussion

One-sided compressions, complete fusions, and compression and fusion were the most common vertebral body anomalies found. The distribution of the anomalies along the vertebral column varied according to the malformation type. Mainly two vertebral segments were affected: the first and the last caudal vertebrae. Different types of compressions in the vertebral bodies were predominant in the transition between the abdominal and caudal regions. This region may have a high mechanical load due to the swimming musculature and flexion of the vertebral column in different fish species (Fjelldal et al. 2021). As suggested by Witten et al., 2005, the combination of disuse and mechanical overload may contribute to the development of compressed vertebrae. Complete fusions affected mainly the caudal-most vertebrae. According to the number of deformed vertebrae, turbot that presented more than ten deformed vertebrae were slightly shorter or wider, although not significantly. However, they had a significant smaller mean ratio StL/BW (1.22) compared to D1-5 (1.31) and D6-10 (1.30) groups ($p<0.001$); although such differences in the proportions of the animals may be sometimes difficult to assess with the naked eye.

Conclusion

The results of this study highlight the presence of vertebral body anomalies in farmed turbot with a very particular distribution along the vertebral column, depending on the malformation type. The presence of several anomalies in the same turbot seems to maintain the external biometric values, except the ratio StL/BW in fish with more than ten deformed vertebrae. Besides, the anomaly profile may have a low impact on turbot marketability. This work could be useful to compare with studies on the anomaly profiles of other flatfish species, for which malformations still represent a problem for its intensive production.

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Acknowledgements

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COMPARATIVE ACCLIMATIZATION AND REPRODUCTIVE MANAGEMENT OF TWO MULLET SPECIES *(Chelon labrosus* AND *Liza aurata*)

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Abstract

The reproductive management of two mullet species (*Chelon labrosus* and *Liza aurata*) is assessed from its capture (2018 for *Liza aurata*, 2019 for *Chelon labrosus*) to the final obtention of viable spawns 4 and 3 years later, respectively. From 60 *Liza aurata* broodstock, acclimated in 3 tanks of 1 m3, 11 natural spawns were obtained from November 2021 to January 2022. From 54 *Chelon labrosus* broodstock acclimated in 3 tanks of 10 m3, 3 induced spawns were obtained in April 2022. From each species, viable eggs and larvae have been obtained under controlled conditions, opening new perspectives to increase the culture of these species.

Introduction

The Mugilidae family presents a great potential for the sustainable diversification of aquaculture due to their eurythermal, euryhaline, and low trophic nature. However, the culture of these species continues depending on fry collection from the wild, which produces high mortalities (Crosetti and Blaber, 2015). In this respect, establishing adequate protocols for wild broodstock collection, acclimatization, and reproductive management is the first step to successfully closing the biological cycle in captivity.

Materials and methods

Fishing and acclimatization

Adult animals were fished and transferred to open sea-water system tanks in the GIA-Ecoaqua facilities (Canary Islands, Spain). One month after acclimatization, animals were anaesthetized with clove oil (50 ppm) and individually pit-tagged. Species genetic corroboration was carried out from caudal fin samples through Polymerase Chain Reaction (PCR) of the 5s-rDNA gene and by subsequent sequencing analyses according to Imsiridou et al. (2007).

Reproductive management

Sex identification of broodstock was carried out before the beginning of the natural spawning season of each species. Males were identified through abdominal massage, while animals negative for this test were gonadally biopsied using a 1.3-mm-internal-diameter catheter, to later visualized the sampled material under a stereoscope. For all the period, both species were fed daily with a commercial organic feed for marine fish (Aller Blue organic, Aller Aqua Group, Denmark). Fish were maintained under natural photoperiod and temperature.

Liza aurata broodstock was maintained untouched through the spawning season, while the spawning of *Chelon labrosus* was hormonally induced in mature specimens following the protocol described by Vallainc et al. 2021.

Table I. Culture conditions and mean weight of mullet broodstock.

	Liza aurata	*Chelon labrosus*
Male/Female ratio	2:1	1.4:1
Stocking density (Kg/m^3)	7	3
Mean female weight (g)	525±90	1626±530
Mean male weight (g)	366±79	1357±250

(Continued on next page)

Results

Fishing and acclimatization

After trying various fishing techniques, *Liza aurata* adults were fished by fishing nets at sunset, while *Chelon labrosus* adults were fished with a fishing rod during the daylight.

As acute haemorrhagic and ulcerative lesions were observed (>50% of the population) during the first week after capture, at arrival the animals were subjected to a preventive enrofloxacin treatment (10 mg/Kg in single intramuscular injection), and to a formaldehyde bath the next day (100 ppm, one hour bath).

Reproductive management

From *Liza aurata* broodstock, 11 natural spawns were obtained 4 years after acclimatization, from November 2021 to January 2022. From *Chelon labrosus* broodstock, 3 hormonally induced spawns were collected in April 2022. The fertilization rate ranged from 0-57.5% in *Liza aurata* spawns, and from 0-5.35% in *Chelon labrosus*.

Discussion and conclusions

Both mullet species are sensitive to the stress of capture and transport, so acute skin damage and ulcerative processes were observed during the first weeks of acclimatization. For these reasons, careful handling and preventive treatments such as formol baths are strongly recommended at arrival. After this period, mullet broodstock adapted well to captivity and dry feed, even obtaining natural spawns in the case of *Liza aurata*, in concordance with previous experiences with this species (Quirós-Pozo et al., 2022, unpublished results). For *Chelon labrosus*, despite achieving the state of final oocyte maturation (FOM) naturally, no spawns were obtained spontaneously, in concordance with previous experiences with this species (Besbes et al., 2020), so hormonal induction treatment was applied as described by Vallainc et al. (2021) with successful results. Although the low fertilization rates, robust larvae and juveniles have been obtained, suggesting the feasibility of culturing both species under controlled conditions.

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EFFECT OF BIO-ENZYMATIC PREPARATION ON WATER AND SEDIMENT QUALITY IN PONDS

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Introduction

At present, there are approximately 24 000 fishponds in the Czech Republic. Decades of inappropriate management at the watershed, excessive erosion of arable land and increased fish production have led to the accumulation of sediments in ponds. In the Czech Republic, a third of ponds show an excessive load of sediments. Accumulation of sediment can lead to a reduction in depth, depletion of dissolved oxygen, accumulation of nutrients, and thus limiting biological, ecological functions, and fish production. The removal of sediment from ponds is necessary for fishpond maintenance. One of the options is the mechanical removal of sediment. However, due to economic reasons, people are looking for other methods of reducing the excessive accumulation of sediments.

In our study, we used the PTP Plus (Baktoma Ltd.), a mixture of bacterial spores and enzymes that should decompose the organic content of sediments in ponds. It is a concentrate of spores and endospores of specially selected and targeted breeding strains of native soil bacteria. Shortly after the introduction of the product into the aquatic environment, spores and endospores should revive and feed the organic sediment. According to the manufacturer, this should result in the reduction of organic deposits, phosphorus and chlorophyll–*a* content in water, while oxygen concentration should increase.

Materials and methods

Monitoring of the physical and chemical parameters of both the water and sediments took place from April to October 2016 and 2017 in three fishponds: BOH I (1 ha), BOH II (0.8 ha) and BOH III (0.6 ha) in the Olomouc Region (Czech Republic). In two of the ponds, bacterial product PTP Plus (Baktoma) was repeatedly applied from May till October 2017 according to the instructions from the supplier - BOH II (recommended dosage) and BOH III (doubled dosage). Basic hydrochemical parameters (dissolved oxygen concentration, water temperature, pH, conductivity and transparency) were measured once a month. Water samples were collected, and the following parameters were analyzed: COD, BOD, total nitrogen and phosphorus, ammoniacal nitrogen, nitrites, nitrates, phosphates, chlorides, acid neutralizing capacity, calcium and chlorophyll–*a*. The surface layer (0–15 cm) of the sediment was collected using an Ekman-Birge grab. The dry mass of the sediment samples was determined and aqueous leachates from fresh samples of sediment were prepared. Basic available nutrients (N, P), COD and organic substances were determined in aqueous leachates. Soil extracts were prepared from dried samples of the sediment using Mehlich III and aqua regia. Amount of the available phosphorus and calcium was determined using the extracts.

Results and discussion

In order to evaluate the possible effects of the PTP Plus preparation, the water and sediment quality in 2016 (before the PTP Plus application) and in 2017 (after application) were compared. Significant difference and increase in conductivity, and the amounts of calcium and chlorides in the water were recorded. In the case of the sediment, a significant difference between years was recorded in some of the parameters (COD, nitrogen, phosphorous, calcium), but not in every pond. Different climatic conditions of each year probably had a major impact on the significant changes in water and sediment quality during the particular years of monitoring. The occurrence of submerged and free-floating macrophytes in 2017 could also have affected the difference in water and sediment parameters between the studied years. Results of the non-metric multidimensional scaling of ponds based on physico-chemical parameters of sediment showed differences among ponds, but did not show year-on-year changes, indicating different sediment conditions of each pond. The expected reduction of the share of organic matter in the sediments after the application of the product was not confirmed in our study. Our results confirm the findings of other authors who state that the effect of the addition of bacterial mixtures into ponds on the improvement of the water and sediment quality is low.

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SOME INSIGHTS INTO FATTY ACID AND ENERGY METABOLISM OF A COLDWATER PERCH SPERM: NUTRITION & KINETICS

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Introduction

Eurasian perch (*Perca fluviatilis*), a temperate but eurythermal (CT_{max} 30-32°C) fish species, is a potential candidate for freshwater aquaculture diversification in Europe. It is a freshwater, carnivorous (piscivore, insectivore, facultative planktivore) fish species, that have evolved feeding on primary consumers of the aquatic food web. As such, their nutritional bioenergetics, e.g., relatively poor carbohydrate tolerance, low endogenous EPA/DHA biosynthesis, higher amino acids, and long-chain >C20 PUFA requirement, closely resembles that of higher trophic level fishes like salmonids. Over the past, there has been some good progress in Eurasian perch broodstock maturation and seed production – including studies on sperm/egg quality, handling, storage condition, etc. Albeit some previous nutritional characterization (mostly fatty acids, and nucleotides) of their gametes, their basic cellular nutritional bioenergetics mechanisms (*i.e.,* relative stoichiometries or turnover of nutrients and energy) have not been fully understood. Here, we have taken *P. fluviatilis* spermatozoa as a model to elucidate some probable mechanisms behind a short burst of motility. Elucidation of these mechanisms is even more important for temperate percids like Eurasian perch, which reproduce (maturation → external fertilization) in metabolically difficult, cellular thermal environments (cold water around 12-14°C). Understanding the strategies in such conditions might help in tailoring captive broodstock nutrition in a better way and open novel biotechnological applications.

Materials and Methods

Pond reared Eurasian perch *Perca fluviatilis* adults (body weight 172.1 ± 16.2 g; age 3+) fed on natural food were obtained from Nove Hrady region (541 m asl), Czech Republic. Harvested fish were transported to an indoor RAS facility in Ceske Budejovice (7°C) and captive maturation was induced by a combination of photo-thermal and hormonal manipulation in April [1]. Males were stripped and selected (initially 15 then 10 hand-picked fulfilling >90% sperm motility). Sperm was collected using 5 ml syringes and avoiding urine or blood contamination. Received sperm in centrifugation tubes were always handled in ice slurry bath (4-6°C). Sperm concentration was assessed in Bürker cell hemocytometer and sperm kinematics were assessed using negative phase-contrast microscope equipped with 20x lens, and uEyecockpit software, set at 25 frame per second [2]. Fresh samples from 10 good males were divided into batches: (a) freshly frozen at −80°C ('control'), (b) test-tube activation around 10°C and freezing −80°C ('treatment'). Treatment was done (1 sperm: 39 diluent) by an activation solution [3]. Biochemical analyses included fatty acids (n=21, C14-C22), peroxidation products (malondialdehyde), nucleotides (IMP, AMP, ADP, ATP, hypoxanthine, inosine, adenosine), and lactate [4]. Fresh sperm were analyzed as original matter. Whereas frozen treatment' was thawed and centrifuged (4°C, 8000 rpm, 10 min) to extract sperm pellet (used for analyses). Relative stoichiometries of fatty acids and nucleotides were derived from the quantitative data (fatty acids and nucleotide content per unit weight of sample) and then also verified from semi-quantified data (relative percentage of total fatty acids and nucleotide per billion spz). Apparent desaturase and elongase activity were estimated by product-to-precursor ratio [5]. Metabolic losses or nucleotide degradation was assessed by K-value change [4] and cellular bioenergetics by established nucleotide balances [6].

Results

Motility parameters: Among studied males, sperm concentration was variable 37.7±8.3 × 10^9 spermatozoa ml^{-1} (CV 22%). Representative results from 10 hand-picked males fulfilling a motility percentage of 90-100% suggest an average motility duration of 30.6±3 seconds (CV<10%). Timepoint of 60 seconds ensured termination of sperm motility. The active spermatozoan had a curvilinear velocity of 168.8±33 $\mu m\ s^{-1}$ (CV 19.7%), straight-line velocity 76.4±33.6 $\mu m\ s^{-1}$ (CV 44%), average path velocity 115.5±41.9 $\mu m\ s^{-1}$ (CV 36.3%), and linearity 62.8±5.9% (CV 9.4%).

Fatty acid metabolism: Like in higher vertebrates and nervous (brain) tissues, spermatozoon at its entirety is mainly composed of a ω-3 LC-PUFA docosahexaenoic acid (DHA, C22:6n3 ~31% of total fatty acids) followed by saturated fatty acid, palmitic acid (PA, C16:0 ~18% of total fatty acids); making up half of the sperm lipidome. While DHA is known to impart structural flexibility and fluidity required to achieve sperm kinetics, the PA serve as an energy source. The palmitic acid: PUFA ratio changed ($p<0.05$) at quiescent versus exhausted stage; suggesting at quiescent state PA served as energy substrate while post-activation PA were desaturated and elongated to PUFAs to supposedly reinforce the sperm kinetics. Apparent activities of Δ9 desaturase (*scd2* gene), bi-functional Δ5/Δ6 desaturase (*fads2*), Δ4 desaturase (*fads2* or purported *degs2*), elongases (*elovl6*, *elovl5, elovl2*) were hinted in both quiescent and post-activation state.

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There might be a gear-up mechanism (1st gear: 'SFA → MUFA' to 2nd gear: 'MUFA → PUFA') supposedly involved when spermatozoon activates from quiescent state. Expression of *scd2* and *elongase6* switched opposite to each other ($p<0.05$), when going from quiescent to post-activation state. *Elovl5* (responsible for C18 to C20 elongation) and Δ4 desaturase (responsible for EPA to DHA conversion) suggested the highest apparent activity in perch spermatozoa, with a higher average expression in post-activation. DHA relative to other fatty acids in exhausted spermatozoa was ~14% higher than in quiescent state. There was no significant change in malondialdehyde content (lipid peroxidation product) between quiescent and post-activation state; values were as low as in fresh fish flesh (internal standard).

Energy metabolism: As expected, degradation (metabolization) of nucleotide increased ($p<0.05$) from quiescent to post-activation stage. Net retention of nucleotides (100- Kvalue %) in quiescent stage was ~63% (pre-store) while it dropped ($p<0.05$) to almost half (~31%) in post-activation. Indeed, the nucleotide degradation was not via free radicals or lipid peroxidation, as malondialdehyde were almost unchanged (above). ATP:ADP ratio was low (0.21: 1; indicating glycolytic cellular metabolism) in quiescent stage and high post-activation (0.71: 1; indicating a switch to oxidative cellular metabolism). It also suggests suppressed mitochondrial function in quiescent stage and higher during post-activation. Higher mitochondrial function corresponded with higher 'adenylate energy charge' post-activation. Adenylate kinase reaction ($2ADP \rightleftharpoons ATP+AMP$), which occur in mitochondria and flagellar axoneme, occurred almost close to equilibrium (0.5-1.0: 1.0) in quiescent stage; but equilibrium was lost, and forward reaction occured post-activation (1.3-3.9: 1.0). Glycolytic metabolism was suggested to be suppressed in post-activation, while fatty acid synthesis continued. These mechanisms fit well with achieving a short burst of motility in cold water.

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TOWARD SEA CUCUMBER PRODUCTION AND IMTA APPLICATIONS IN EUROPE: PROGRESS, PROBLEMS AND OPPORTUNITIES

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Introduction

There is an increasing focus on the effects of organic wastes produced by intensive aquaculture, and on solutions to reduce the polluting impacts of European marine farms worldwide. Integrated Multi-Trophic Aquaculture (IMTA) is a new generation aquaculture that allows for the reduction of environmental impacts, higher profits and the diversification of commercial production. This integrative approach, in fact, offers a natural means of encouraging nutritional recycling within aquaculture farms, simulating a natural community through the employment of extractive species with a low trophic level and high market value. In this context, sea cucumber as deposit feeders species could represent promising candidates for co-culture in IMTA, considering their feeding habits and their high market value. However, although their ecological role in many marine habitats and their feeding behaviour seems highly promising compatible with extensive integration, there are only few investigations available regarding the co-culture of European sea cucumbers in productive systems (MacDonald et al., 2013; Tolon et al., 2017; Neofitou et al., 2019; Grosso et al 2021; Bastien Sadoul et al., 2022; Cutajar et al., 2022).

In this context, aquaculture could offer a sustainable alternative to the exploitation of the wild population, to meet current market demand and at the same time could promote the waste bioremediation nearby productive areas. With regard to European sea cucumber species aquaculture, a consistent body of research literature has been produced in the last few years, paving the way for the artificial reproduction of many European species by developing a spawning induction methodology and larval rearing protocol (Domínguez-Godino et al 2015; Domínguez-Godino et al 2018; Rakaj et al 2018; Rakaj et al., 2019; Laguerre et al., 2020; Magdy et al.,2021; Schagerström et al., 2022). Nevertheless, there are still some challenges facing sea cucumber aquaculture that need to be overcome for future aquaculture development. This brief keynote present the up-to-date research progress and problems and future opportunities for European sea cucumbers in aquaculture.

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A TRANSCRIPTOME GENERATED FROM HYBRID-CORRECTED PACBIO LONG-READS IN ATLANTIC SALMON PROVIDES 71000 HIGH-QUALITY FULL-LENGTH SEQUENCED MRNAS, AND IDENTIFIES THOUSANDS OF NOVEL SPLICE VARIANTS

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Introduction

Atlantic salmon (*Salmo salar*) is a major species produced in world aquaculture [1] and an important vertebrate model organism for studying the process of rediploidization following whole genome duplication events (Ss4R, 80 mya) [2]. The then current *Salmo salar* transcriptome was largely generated from genome sequence based in silico predictions supported by ESTs and short-read sequencing data [3]. However, recent progress in long-read sequencing technologies now allows for full-length transcript sequencing from single RNA-molecules. A full-length protein coding transcriptome from a species (the CDS as well as the 5'- and 3' UTRs) and its repertoire of splice variants is an essential resource to reliably annotate protein coding transcripts and understand how such structural variants impact disease and economical important traits in farmed animals [4]. The aim of this study was to generate a high-quality full-length transcriptome resource for Atlantic salmon, based on sequencing of samples from tissues and conditions of broad interest to salmon research.

Material and Methods

Trandcriptome sequencing was carried out in liver, head-kidney and gill materials, sampled before and during smoltification as well as after sea-water transfer. Head-kidney from fish challenged with a viral infection was also included. RNA extraction was performed using the Qiagen RNeasy Mini Kit according to the manufacturers protocol. All samples were sequenced both with PacBio long-read SMRT sequencing and Illumina 150bp paired-end sequencing. A pipeline for data-analysis was developed based on Iso-seq sequencing of long-reads on the PacBio platform (HQ reads) [5] followed by error-correction of the HQ reads by short-reads from the Illumina platform using the LoRDEC algorithm [6]. This was followed by in-house developed filtering steps, and multiple clustering steps using cDNA_Cupcake, Cogent and CD-hit. Finally, functional annotation and classification of transcripts (splice variants or different genes) was performed based both on the reference genome and Blast searches against other chordates using the SQANTI [7] and OmicsBox [8] software package respectively.

Results

The pipeline successfully processed more than 1.5 million long-reads and more than 900 million short-reads into error-corrected HQ reads. A surprisingly high percentage (32%) represented expressed interspersed repeats, while the remaining were processed into 71 461 full-length mRNAs from 23 071 loci. Each transcript was supported by several single-molecule long-read sequences and at least three short-reads, assuring a high sequence accuracy. On average, each of the 23071 genes were represented by three isoforms (splice variants). More than 80% of transcripts were assigned Gene Ontology terms. Comparisons to the current Atlantic salmon transcripts in the RefSeq database showed that the long-read transcriptome validated 25% of all known transcripts (current GenBank reference mRNAs), while most of the remaining full-length transcripts were novel isoforms of known genes, with 566 transcripts putatively stemming from novel genes. A comparison to the current genome assembly indicates that the long-read transcriptome greatly improves transcript annotation and may provide long-read linkage information useful for improving the genome assembly. Thousands of transcripts were from genes or splice-variants expressed in an organ-specific manner demonstrating that hybrid error-corrected long-read transcriptomes may be applied to study genes and splice-variants expressed in certain organs or conditions (e.g. challenge materials).

Conclusion

At time of writing, this study represents the single largest contribution of high-quality full-length mRNAs in Atlantic salmon. The results will be of great value to salmon genomics research, and the pipeline outlined may be applied to generate additional de novo transcriptomes from other tissues in Atlantic Salmon or be applied for similar projects in other species.

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CHARACTERIZATION AND INCLUSION OF VEGETABLE BY-PRODUCTS IN SUSTAINABLE AQUAFEEDS. THE EXAMPLE OF BANANA BY-PRODUCTS

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Introduction

The evolution of the different raw materials used in animal feed production over the last 30 years indicates an increase in the use of cereals, with soya, corn and wheat leading the way, following the 2001 crisis, and a reduction in the use of the animal meal. One of the utilities of by-products from crops have been to use them for animal feed (Federici *et al*., 2009), as fodder.

In the case of bananas, this traditional use has been naturally occurring throughout the world and in the Canary Islands (Padam *et al*., 2014).

However, since the early 2000s, authors have been pointing out the potential of by-products from the agro-industrial sector as functional ingredients for the human and animal food industry (Schieber *et al*., 2001). This has led to the search for new uses of by-products from other industries, be it food, alcoholic beverages or bioethanol production, especially in the last twenty years.

Material and Methods

Biochemical and functional characterisation of two banana by-products, pseudo-stem (BP) and flower (BF) were carried out. Based on these results, consecutive trials were developed for the global valorisation of both products in juveniles of two species of high interest in aquaculture, tilapia and sea bass. Three studies were performed for each of these species, one trial with BP, a second with BF and a third with organic extracts and BF residue, in which the bioactives responsible for the beneficial effects of banana blossom would be present. Different growth parameters, biochemical and histopathological characteristics, oxidative status and immune parameters of both species were evaluated.

Results & Discussion

Treated BP reduced the lipid content in tilapia and modified the fatty acid profile, while in sea bass the lipid content was not affected. BF increased the amount of n-3 fatty acids in tilapia, while it did not affect sea bass. BP improved the oxidative status of the seabass liver and BF improves stress response parameters and the cellular response to simulated infection in seabass. Therefore, it should be noted that both BP and BF are both novel by-products of banana production, with potential for inclusion in feed for aquaculture and species of different trophic levels, with a high content of non-extractable polyphenols in BF, which positions it as a functional ingredient of interest (Ramírez-Bolaños *et al.,* 2021). Further studies on the effect of different processes on raw materials, mainly on BF, to increase the percentage of inclusion in feed would be of interest based on these first studies with pre-processed products.

Based on the results, treated BP can be included in tilapia diets for up to 5% and untreated BP can be included for up to 8% in sea bass. Untreated BF can be included at 3% and 6% in tilapia and sea bass diets, respectively.

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DEVELOPMENT AND EVALUATION OF GROWTH MODELS FOR NILE TILAPIA

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Introduction

In order to achieve optimal performance in aquaculture, it is important to maximize growth while reducing costs and ensuring the sustainability of production activities. To do so, feeding strategies need to be optimized through careful daily estimation of the total biomass. Due to the impracticality of daily measurement of fish biomass, it is crucial to understand in an objective and practical way, the growth patterns and their underlying mechanisms, to enable (for example) biomass estimation between measurements. Traditionally, empirical mathematical models (e.g. RGR, TGC, FCR) have been used in aquaculture to predict growth due to their simplicity and ease of parameter determination. However, such models often ignore important aspects, such as feeding information (e.g. feed quantities properties), and/or lack clear physical/biological support, being inadequate as general context-independent prediction models. In academic contexts, other alternatives have been suggested, such as the use of bioenergetic models or nutritional models (e.g., energy-protein models). These types of models take into consideration feed quantities and (at least some) properties, which allows them to provide more precise growth predictions, as well as other important information (e.g., environmental impact due to waste and excretion losses). However, there is still some scientific and technical questions regarding the implementation of these models, which perhaps hampers their more widespread adoption in commercial settings.

In this work, tilapia growth data were collected and used to compare different combinations of models and calibration methods, using objective criteria based on cross-validation, to determine the best combination for this species.

Materials and methods

Nile tilapia growth data were collected from 27 publications, covering sizes from 0.51 g to 457 g. Primarily, an exploratory analysis of the data was performed to see how relevant responses were affected by different factors (e.g. body weight, temperature, feed intake, digestible protein intake, digestible energy intake). Bioenergetic and energy-protein flux models were then developed and tested with different calibration methods. Several “model + calibration method” combinations were evaluated through different cross-validation methods (leave-one-out cross-validation, repeated k-fold cross-validation with k=10, k=5 and k=2), to objectively evaluate their predictive capacity. This evaluation was performed considering both qualitative (diagnostic plots) and quantitative aspects (calibration and cross-validation RMSE, MAPE and CRM).

Results

Exploratory analysis of the data suggests a linear relationship between digestible protein intake and protein gain, and between energy intake and energy gain.

Considering the results of cross-validation, nutritional models, like the EP model (energy-protein flux), appear to provide better predictions than the simpler bioenergetic models, with the EP models showing cross-validation errors (e.g. MAPE) on the order of ≈ 8 % and ≈ 14 %, whereas the bioenergetic model had ≈ 13 % and ≈ 14 %, for the body weight and body composition predictions, respectively (in a 5-fold cross-validation). Regarding the calibration methods, an important finding was that models have better growth predictions when calibrated assuming the standard body weight exponents of 0.8 and 0.7 for maintenance costs (energy and protein, respectively), than when the estimated exponents are used.

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Discussion and Conclusion

The results regarding the linear relation between digestible energy intake and energy gain are consistent with what has been previously demonstrated by Lupatsch et al. (2003) for gilthead seabream, but in disagreement with the quadratic relation that Van Trung et al. (2011) reported for the specific case of Nile tilapia. The relation between digestible protein intake and protein gain was also linear, which disagree with the quadratic relation that Van Trung et al. (2011) suggested for Nile tilapia.

The fact that EP models have a better predictive ability than bioenergetic models shows that protein intake is an important component to consider when estimating growth in Nile tilapia. Perhaps, at least in part, this effect can be explained by the generally low protein content in tilapia feeds, which suggests that growth is protein-dependent.

The energy and protein maintenance costs exponents for body weight seem to show better results when they are fixed, using the standard values of 0.8 and 0.7, respectively (Clarke & Johnston, 1999), or assuming isometry (exponents equal to 1), rather than estimated from the data.

Overall, in this study, the use of evaluation methods that consider cross-validation error metrics enabled the clarification of important scientific (e.g., best model to predict growth) and technical (e.g., best calibration method) questions, providing a meaningful contribution to a more widespread adoption of highly-predictive nutrient-based fish growth models.

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THE HORIZONTAL INTEGRATION OF A SHELLFISH FARM IN A BROADER BUSINESS MODEL

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The demand for Eastern oysters over the last two decades has increased year after year. This steady continuous growth has created opportunity not only for oyster farmers and other businesses immediately supporting the shellfish aquaculture industry, such as gear and transportation, but it has also created opportunities just outside the shellfish aquaculture market. Opportunities to reach retail markets, such as restaurants and open-air markets, with their product and integrating into establishing those market on their own.

In order to meet those markets directly Matunuck Oyster Farm has horizontally integrated in each stage of the cycle of the eastern oyster (Crassostrea virginica). Matunuck Oyster Farm was established in 2002 as a 1-acre oyster farm with one employee. Now, 20 years later, that business has expanded to five additional businesses with over 200 employees, each business having the farmed Eastern oyster being a central, integral part of the business. Each of the new businesses support the farm, and the farm supports each business, creating more financial stability for the oyster farm.

The growth of Matunuck Oyster Farm into the six other sectors has been organic and determined by company needs. In addition to selling oysters to wholesalers, we expanded by offering different products such as Bay Scallops. We then started selling oyster seed to other farmers, selling at open air markets, and established Matunuck Oyster Bar, a popular restaurant overlooking the shellfish farm. This led to the establishment of Matunuck Organic Vegetable Farm, Matunuck Marina, Matunuck Shellfish Hatchery Research and Innovation Center and most recently a retail market in our adjacent downtown area of Wakefield, Rhode Island.

Perry Raso, Founder and Owner of these businesses, will discuss synergies between each of the businesses and how each business has strengthened and added value to each other.

THE EFFECT OF CLIMATE CHANGES IN SURVIVAL AND BEHAVIOUR OF EUROPEAN CLAM (*Ruditapes decussatus*)

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Introduction

Climate changes represent a potential limiting factor to aquaculture, directly interfering with survival and behavior of bivalves. Severe fluctuations in temperature along with periods of heavy rainfall or periods of drought that significantly change the salinity[1], can promote physiological stress of bivalves, resulting in changes in physiological and behavioral responses and, in extreme cases, leading to high mortalities[2,3].

European clam (*Ruditapes decussatus*) represents one of the most relevant emergent bivalve species from the aquaculture sector in Europe with high economic value. Considering the predicted climate changes and given the economic and ecological importance of *R. decussatus*, the current study was designed to evaluate the effects of different combinations of salinities (0-40) and temperatures (5-35°C) on mortality and feeding behaviour of juveniles and adults of *R. decussatus*.

Material and methods

Juveniles and adults of clams *R. decussatus,* captured in Ria Formosa lagoon, were exposed to combined ranges of temperature and salinity. Considering the annual temperature and salinity variations in this coastal area over the last years, it was established a control temperature range between 15°C and 23°C, inclusive, and salinity 35.

Three and two trials of 120 h were performed for adults and juveniles, respectively.

Mortality and feeding behaviour were evaluated and registered every 24 hours. Individuals were considered dead every time they were unable to close their valves after mechanical stimulus[4], being then removed from the beakers. Feeding behaviour was evaluated through the absence or presence of faeces, scored as 0 or 1, respectively. Median lethal time (Lt50) and median lethal temperature (LC50) were calculated.

Results

Low salinities and high temperatures caused higher mortality rates, in both juveniles and adults, after 120 hours of exposure. In the control temperature range (15°C - 23°C), juveniles revealed to be more sensitive to the salinity variations. As salinity increased, juveniles' mortality decreased. In adults, the mortality rate reached 60% of the individuals at salinities lower than 15. Above the upper limit of the control temperature range (23°C) and salinities inferior to 15, the observed mortality was almost always extremely high, for both size classes. In more saline waters, juveniles seemed to be more resistant than adults, mainly at extreme temperatures (32°C and 35°C), where occurred 100% of mortality of the adult population. For salinities equal or below 15, LC50 varied between 20.28°C and 22.29°C for juveniles and adults, respectively, for salinities higher than 15, LC50 was set at 31.08°C for juveniles and 30.08°C for adults. In temperatures higher than 23°C and salinities less or equal to 15, Lt50 substantially decreased as temperature increased, with juveniles more sensitive to higher temperatures (32°C and 35°C). At higher salinities, juveniles seemed to be more tolerant than adults, however, the mortality of 50% of adults' population would never be expected to occur below 30 hours of exposure.

Feeding behavior seemed to be highly influenced by salinity, regardless the temperature, for both juveniles and adults. At salinities lower than 15, it appeared that individuals did not feed. From this point and at control temperature range, the feeding behavior of juveniles and adults was maximum. Out of this range, juveniles were less active at lower temperatures and more active at extreme temperatures. On the other hand, adults showed the opposite pattern. Mortality revealed a positive correlation with temperature while salinity had a strong positive influence on feeding behaviour.

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Discussion

Our results suggested salinity 15 as “turning point” below occurred a change in mortality and feeding behavior, which is in accordance with previous studies for venerid species [5,6]. Salinity fluctuations have a direct impact on feeding behavior, while temperature has a major impact on survival of clams. Low salinities and high temperatures are lethal to *R. decussatus.* Juveniles are less resistant to the increase of temperature in less saline environments, however, more resistant than adults to extremely high temperatures under more saline conditions.

Considering the predicted climate changes scenarios, juveniles will die faster when exposed to low salinities and high temperatures, consequences of heavy rainfall and heatwaves, never reaching the adult stage, thus compromising reproduction and consequently, bivalve recruitment. The failure in bivalve recruitment will have a strong negative impact on the economic sector due to the decrease in the available biomass for harvesting.

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INVESTIGATING THE CHEMICAL NATURE OF MALE PHEROMONE IN PACIFIC OYSTER, *Magallana gigas*

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Introduction

Chemical communication is essential in several aspects of the life cycle of aquatic organisms. In the case of broadcast spawning species, such as oysters, pheromones play a crucial role in spawning synchronization. It is well known that oysters can be induced to spawn by thermal shock, however, females usually need an additional stimulus to trigger spawn. In general, this is achieved by adding conspecific sperm to the water. This way, sperm carries a chemical signal that stimulates/induces spawning in females: a spawn-inducing pheromone (SIP) (Galtsoff, 1938; Taylor et al., 2018). In this study, we investigated the chemical nature of male SIP in the Pacific oyster, *Magallana gigas* through an electrophysiological technique, the 'electro-osphradiogram'.

Material and methods

Oysters were induced to spawn by thermal shock (15°C - 29°C, at intervals of 1h). Once males started to spawn, they were transferred to a beaker with 200 ml of charcoal-filtered seawater to obtain natural spawning sperm (NS). After 15-20 minutes, males were opened, and sperm was obtained through incisions in the gonad – stripped sperm (S). In a first approach, "NS" sperm and "S" sperm from each male were centrifuged (10 min, 7200 rpm). The chemosensitivity of the oyster osphradium to total sperm and sperm fractions (supernatant and pellet) of both "NS" and "S" sperm (fresh and frozen) was assessed through electro-osphradiogram (EOsG), a D.C. field potential. Then, fresh and previously frozen (-20°C) NS and S sperm pools, were centrifuged (30 min, 2500 rpm, 4°C). Each supernatant was extracted through solid-phase extraction (SPE) C18 cartridges, and filtrate (aqueous fraction) and eluate (elution with methanol) were collected. Afterwards, filtrate and eluate were assessed for 'olfactory' activity by EOsG. The voltage signal was amplified (x2000 - x20 000) with the low-pass filter set at 30 Hz. The signal was then digitised and recorded on a PC running Axoscope TM software. L-cysteine (10^{-3} M) was used as a positive control (or standard) whereas the natural filtrated seawater was the blank or negative control. The EOsG peak amplitude was measured in millivolts. All recorded responses were blank-subtracted and normalized to the standard (10^{-3} M L-cysteine).

Results and discussion

The method of sperm collection affected the amplitude of the EOsG response; the stripped sperm (S) was more potent than sperm obtained through natural spawning (NS). This may be due to sperm emulsion containing some remains of gonadal tissues resulting from the incisions in the gonad and/or the sperm collected at higher density.

Similarly, when comparing fresh with frozen sperm, the latter evoked a higher EOsG amplitude. This can be explained by the cellular rupture associated with the freezing process releasing additional odorants from the spermatozoa.

In general, the supernatant evoked greater EOsG amplitude than the pellet, and similar to those of total sperm. After SPE extraction of the supernatant, the eluate fraction did not evoke any EOsG response whereas the aqueous fraction – the filtrate – evoked the highest amplitude responses, similar to that of total and supernatant; the majority of pheromonal/olfactory activity is in the aqueous fraction of the supernatant.

As in other marine invertebrates, such as polychaetes (Zeeck et al., 1998a, 1998b, 1998c), the pellet appeared to be pheromonally inactive whereas pheromonal activity was mostly contained in the supernatant and, in the case of this oyster species, is contained in the aqueous fraction of the supernatant. In contrast, in the pearl oyster (*Pinctada maxima*), it has been suggested that the pheromone is a proteinaceous multi-component associated with the sperm membrane (Taylor et al., 2018).

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Therefore, these results suggest that the male pheromone consists largely of (a) polar compound(s). Future work will focus on isolation and identification of the polar compound(s) that trigger females, and other males, to spawn. A successful identification will be an important step towards a future where it is possible to induce oysters to spawn, without the need to sacrifice individuals to obtain sperm emulsions.

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PHYSIOLOGICAL RESPONSES OF RAINBOW TROUT (*Oncorhynchus mykiss*) FED DIETS INCLUDING *Hermetia illucens* PREPUPAE MEAL ENRICHED WITH SPIRULINA (*Arthrospira platensis*)

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Introduction

Over the last decades, the trend in the aquafeed formulation has been characterized by the reduction of fish meal (FM) and fish oil (FO) in favor of more sustainable alternatives. Insect meal has emerged as one of the most promising new aquafeed ingredients. More specifically, the black soldier fly (*Hermetia illucens*) represents an encouraging and increasingly studied insect species for aquafeed formulation due to its proper protein content and an essential amino acids profile similar to that of FM. Furthermore, the *Hermetia illucens* prepupae meal (HM) contains different bioactive molecules like chitin, antimicrobial peptides and lauric acid known for their immunostimulant and/or anti-inflammatory activity. The inclusion of full fat HM in fish diets is still limited by its unbalanced fatty acid profile (rich in saturated fatty acids and poor in polyunsaturated ones); however, it has been widely demonstrated that the HM fatty acid profile can be modified enriching the prepupae rearing substrate with potential sources of polyunsaturated fatty acids (PUFA). In this context, the addition of Spirulina (*Arthrospira platensis*) dried biomass to the insects' growth substrate may represent an interesting way to improve the nutritional value of the prepupae and, thus, of HM. Spirulina is the most common cultured cyanobacteria being produced at commercial scale and it contains high-quality protein and several bioactive components, such as vitamins, minerals, carotenoids, and tocopherols with antioxidant properties. In light of this, the present study was aimed to: (i) improve the nutritional value of HM in terms of PUFA and bioactive molecules by adding *A. platensis* to the insect growth substrate (15% w/w), (ii) formulate two experimental diets characterized by two different dietary inclusion levels (3 and 20%) of "enriched" HM, (iii) investigate the physiological effects of the experimental diets in rainbow trout (*Oncorhynchus mykiss*) juveniles during a 42-days feeding trial.

Materials and Methods

Two experimental diets characterized by 3 and 20% of HM inclusion levels in replacement of FM (named HM3 and HM20, respectively) were prepared starting from a control diet (HM0) formulated with marine (FM and FO) and vegetable ingredients. The three diets were formulated to be grossly isoproteic (50% on dry matter), isolipidic (23% on dry matter) and isoenergetic (25 MJ/kg).

Five hundred forty rainbow trout juveniles (initial weight: 15.28 ± 2.20 g) were randomly divided into nine square fiberglass tanks (in triplicate). Fish were hand-fed on the three experimental diets at apparent satiety for 6 weeks. At the end of the trial, fish were individually measured and liver, pyloric caeca, and distal intestine or whole fish were sampled. Final survival rate was calculated by daily recording of dead fish. For zootechnical performance, weight gain, specific growth rate and Fulton's condition factor (K) were calculated. Histological analyses were performed on liver samples to assess the integrity of liver parenchyma and the degree of fat accumulation, and on distal intestine to investigate the gut health status by means of morphometric evaluation and histopathologic indexes. Molecular analyses were performed to analyze the relative expression of genes involved in stress response, fish growth, fatty acid biosynthesis and inflammatory response. To determinate the macromolecular composition of liver samples, FTIRI measurements were performed. Gut microbiota analysis was performed on whole digestive tract by amplifying and sequencing the V3-V4 region of the bacterial 16S rRNA and sequences were analyzed.

Finally, the effectiveness of enriching insect substrates with bioactive compounds was also evaluated by analyzing fillet fatty acid profile by gas-chromatography.

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Results and Discussion

At the end of the trial, the survival rate of fish was 100% in all the experimental groups and fish growth did not show significant differences among the experimental groups. This result was fully supported by the molecular analysis and was in accord to most of the literature. In the present study, the dietary fatty acids analysis revealed an increasing percentage content of total PUFA, ω3-PUFA, DHA and EPA from HM0 to HM20 diets. As a consequence, in fish, the expression of genes related to lipid metabolism did not show significant differences among the experimental groups. Even carotenoids and total tocopherols showed the same increasing trend.

Interestingly, the fatty acid profile of HM20 trout fillets was the richest in ω3-PUFA, especially in C18:3-ω3, while HM3 contained higher level of DHA only compared to the control group. According to the literature, the dietary inclusion of HM was highlighted by the increasing content of lauric acid (C12:0) revealed in fish fillet.

Intestine plays a key role in nutrient absorption and therefore, evaluating the intestine micro-anatomy and inflammatory markers represents a proper and useful approach when new ingredients are tested. Histological analysis showed that the mucosal fold height was significantly reduced in fish fed HM20 diet respect to control diet; however, no sign of intestinal inflammation was detected in all the experimental groups probably because of the presence of specific bioactive molecules (lauric acid, tocopherols and carotenoids) with anti-inflammatory and antioxidant properties of which the HM20 diet was particularly rich. However, most of the genes involved in the immune response showed an upregulation in HM20 group respect to HM3 and HM0; this result can be related to a possible upcoming intestinal inflammation, not yet macroscopically detectable, or to the decrease in microbiome phylogenetic richness (dysbiosis) observed in fish fed HM diets. Regarding the liver, no differences in the histological architecture and macromolecular composition were observed among groups. In addition, stress markers gene expression did not show differences among the experimental groups. In conclusion, the present study evidenced that lipids (particularly PUFA) from *A. platensis*, tocopherols and carotenoids, were successfully transferred to the fish diets by enriching the insects' growth substrate, demonstrating the suitability of the enrichment procedure proposed. As regards fish fatty acids profile, a dose dependent ω3-PUFA increase was observed from H0 to H20 group. Furthermore, fish welfare was not negatively affected by the experimental diets, while a reduction of mucosal fold height in distal intestine was observed only in HM20 group. Finally, an increased immuno-related gene expression was detected only in HM20 group; this result requires further studies in order to understand if this is a precocious sign of an upcoming intestinal inflammation.

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REARING GIANT FRASHWATER PRAWNS (*Macrobrachium rosenbergii*) IN AQUAPONIC SYSTEMS USING SUSTAINABLE AQUAFEEDS

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Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a widely farmed decapod species worldwide due to its nutritional value, good meat quality and high growth rates. To ensure proper growth and constant productivity, aquafeeds commonly used for this species contain high percentages of unsustainable and expensive marine-derived protein sources like fish meal (FM). To increase aquafeed sustainability, great attention has been given to alternative ingredients like insect meal, that has been shown to be able to replace high amounts of FM in diets intended for farmed aquatic species. Some insect species, such as the black soldier fly (*Hermetia illucens*), beside the proper protein tenor and amino acid profile and the appreciable content of bioactive molecules (i.e. lauric acid, chitin, and antimicrobial peptides) with antioxidant, antimicrobial and immunostimulatory features are able to modulate their nutritional profile, especially in terms of fatty acids, in relation to the growth substrate. On this regard, enriching the *H. illucens* growth substrate with microbial biomass of *Arthrospira platensis* (spirulina), rich in essential amino acids (methionine, cystine, and lysine), polyunsaturated fatty acids, vitamins, minerals, and pigments with antioxidant properties, can represent an efficient method to exploit the beneficial properties of both ingredients. The use of aquafeeds that include ingredients with antioxidant and immune-boosting properties could be of primary importance for species that are frequently subjected to infectious diseases with consequent economic losses, like *M. rosenbergii*.

The present study was aimed to improve the nutritional value of full-fat *Hermetia illucens* meal (HM) by adding 15% w/w of *A. platensis* to the larval growth substrate and then to include (3 and 20 % respect to FM) the "enriched" HM in diets for *M. rosenbergii* juveniles. To further improve the prawns culture sustainability, the 60-day feeding trial was conducted in an aquaponic system (MjEnergy, Treia, Macerata, Italy).

Materials and Methods

Three test diets were formulated to be grossly isoproteic, isolipidic and isoenergetic. A control diet (HM0) containing FM, vegetable ingredients and fish oil was used as basal formulation for the test diets characterized by 3 and 20 % of HM inclusion levels respect to FM (named HM3 and HM20, respectively). At the beginning of the trial, 1971 giant freshwater prawn juveniles were randomly divided into nine aquaponic systems according to the three dietary treatments (in triplicate). Each system consisted of a hydroponic unit for plants cultivation and a prawn tank (total volume of water: 720L). In each hydroponic unit, 15 lettuce (*Lactuca sativa*) seedlings were planted one day before the introduction of prawns. Feeding trial duration was 60 days during which prawns almost triplicated their weight and were hand-fed the experimental diets, at apparent satiety. At the end of the trial, prawns were sacrificed by thermal shock for individual measurements and for excision of the cephalo-thorax for collection of hepatopancreas samples. For zootechnical performances, survival rate, weight gain, percentage of weight gain, and specific growth rate were calculated. Histological analyses have been focused on the middle portion of each hepatopancreas tubule (called B cells zone) since this part includes the mature stage of B and R cells. The relative abundance of B and R cells, as well as the lipid droplets abundance in R cells (as indication of the degree of lipid accumulation), tubule diameter, and the height of the epithelium were measured on 20 randomly selected tubules per section (3 sections per prawn, 15 prawns per dietary treatment). Molecular analyses were performed on hepatopancreas samples to analyze the relative expression of genes involved in prawn molting regulation (*jheh*), enzymatic hydrolysis of chitin (*chit3*), protein digestion (*catL*), stress response (*hsp90*), and immune response (*α2m*).

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Results and Discussion

All the experimental diets were well accepted by the prawns leading to the absence of significant differences among the experimental groups in terms of zootechnical performances. The suitability of the different experimental diets was confirmed also by the histological and molecular analyses on the hepatopancreas, the largest organ in the digestive tract of Decapoda, involved in absorption, digestion, and storage of nutrients as well as in immune response. In the present study, lipid absorption and storage were not affected by the provision of HM diets, as demonstrated by the comparable frequency of R cells and the high lipid droplets abundance among the dietary treatments. In particular, the massive presence of lipid droplets in R cells in all the experimental groups can be associated to the necessity of juvenile prawns to sustain energy-demanding processes like molting. Accordingly, the absence of significant differences in *jheh* gene expression confirms that molting processes were not affected by the different dietary treatments.

This positive *scenario* was evident despite the increasing dietary content of chitin in HM3 and HM20 diets that could impair nutrient absorption due to its complex matrix. On this regard, prawns possess specific chitinases (*chit3*) able to digest chitin since insects are part of the natural diet of *M. rosenbergii*. However, it should be pointed out that, in crustaceans, a reduced bioavailability of dietary nutrients is usually associated to an increasing in B cells population in the hepatopancreas, which are involved digestive processes at cellular level. Accordingly, in the present study, significant differences were evident in terms of B cell relative abundance in HM3 and HM20 groups compared to HM0. This result was fully supported by the expression of *catL* that codifies for the cathepsin L, a protease stored in the digestive vacuole of B cell.

Finally, considering the hepatopancreas health status, no structural alterations were highlighted by the histological analyses and no significant differences were evident in terms of expression of genes involved in stress (*hsp90*) and immune (*α2m*) response, confirming the suitability of the experimental diets used in the present study for prawns' culture.

In conclusion, the present study represents an example of how *M. rosenbergii* culture can be implemented with more sustainable dietary formulations and farming techniques like aquaponic.

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MODULATION OF SKIN AND INTESTINAL MUCOSAL BARRIER FUNCTION IN ZEBRAFISH (*Danio rerio*) FED DIFFERENT POSTBIOTICS AND A PROBIOTIC FROM *Lactobacilli*

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Introduction

Lactobacillus species are among the most widely used probiotics. Despite a plethora of evidence advocating their beneficial effects, there are a number of practical limitations to their industrial application in animal feed. Besides, a growing body of literature examines the nutraceutical potential of inactivated microbial fractions ('postbiotics') processed from specific strains of *Lactobacillus*. Those products are supposed to exert a crosstalk with the host cells and to have positive impact on intestinal barrier and intestine-organs axis. However, only few *in vivo* evidence are available.

The study tested the dietary supplementation with a single proprietary *L. helveticus* HA122 strain with known specific parietal features supplemented either live (probiotic, LHPro) or inactivated (postbiotic; LHPost) as well as with a *L. paracasei* HA-108 postbiotic (LPPost) on the mucosal barrier function of zebrafish used as vertebrate model.

Materials and Methods

The 5-week trial (Plymouth, UK) tested 4 dietary groups in triplicate using wild-type adult zebrafish ($BW_i = 0.52 \pm 0.06$ g; 25 fish/tank) and a basal diet formulated to NRC requirements for cyprinids (36% protein, 8% lipid). Treatment consisted of 1] non-supplemented basal diet (Control), and basal diet supplemented with 2] LH probiotic (LHPro; 20 mg/kg), 3] LH postbiotic (LHPost; 60 mg/kg) or 4] LP postbiotic (LPPost; 350 mg/kg). All microbial products were supplemented at 1×10^6 cells/g feed and consisted of proprietary microbial strains provided by Lallemand SAS (France). Diets were fed at fixed rate of 4% biomass daily over 3 daily rations. At the end of the trial, intestinal and skin histomorphometry (n = 9 fish/ treatment) as well as the intestinal immune response using ELISA, flow cytometry and gene expression analysis (n = 6 fish/ treatment) was assessed. Differences between control and experimental groups were assessed by non-parametric permutation test with significance accepted at $p < 0.05$.

Results

The intestinal lysozyme level (by ELISA) was significantly higher in the LHPost (+38.7%, $p < 0.005$) and LPPost (+33.3%, $p < 0.05$) groups compared to the control; (**Fig 1A**) in line with a significant up-regulation in the expression of *lyz* in the intestine (LHPost: +70.2%, $p < 0.05$; LPPost: +63.2%, $p < 0.05$; **Fig 1B**). The expression of *catL* (coding for cathepsin L) was significantly up-regulated in all treatment groups compared to the control ($p < 0.0001$).

Compared to the control, goblet cell coverage (GCC) was significantly increased in the LPPost group (intestine: +39.8%; skin: + 54.0%; $p < 0.05$; not shown) and goblet cell density (GCD) was increased in the LHPost group (intestine: +23.1%; $p < 0.05$, not shown). In the intestine, the abundance of intraepithelial leukocytes (IEL) was also significantly higher in all supplemented diets (LHPro: + 23.1%; LHPost: + 43.4%; LPPost: + 23.1%; $p < 0.001$; **Fig 2**).

Flow cytometry analysis of the intestinal tissue (**Fig 3**) revealed healthy (> 0.9) and consistent ratios of $CD4^+$ to $CD8^+$ cells across all treatments; alongside with significant elevations of $CD4^+$ (+ 62.9%, $p < 0.05$) and $CD8^+$ (+ 63.7%, $p < 0.01$) cells in the LPPost group compared to the control.

Conclusions

The study identifies the potential of postbiotics to strengthen barrier functions at two levels. Firstly, by promoting the goblet cell population that could lead to better antigen sensing via formation of goblet cell-associated antigen passages (GAPs). Indeed, actively secreting goblet cells can take up antigenic material for further processing by dendritic cells and tissue specific macrophages for presentation to mobilise adaptive T-cell and B-cell responses (Johansson and Hansson, 2016). Secondly, by stimulating innate humoral defences and increasing the presence of innate immune cells in the submucosa of the intestine as shown by elevated levels of lysozyme and IELs, CD4+, CD8+ cells respectively. Together these findings demonstrate the potential of bacterial postbiotics to support intestinal and skin immunity and warrant further research on their application in target species.

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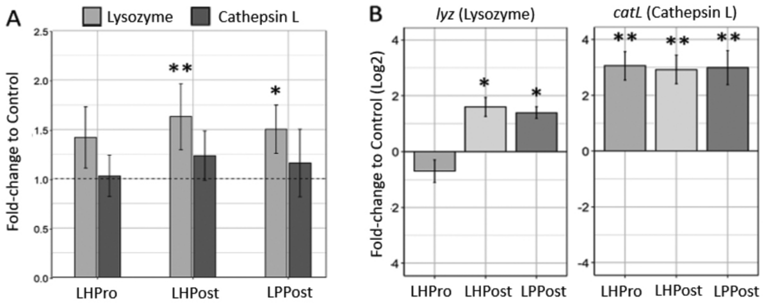

Fig 1. **A)** ELISA showing increased lysozyme level in both postprobiotic groups. **B)** Upregulation of *lyz* and *catL* gene expression across test diets compared to the control group. Mean ± SEM; n = 6, **p* < 0.05; ***p* < 0.005).

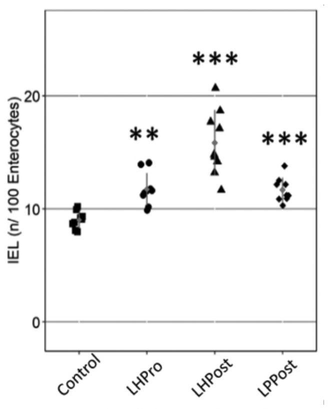

Fig 2. Significant elevations in IEL abundance in all experimental groups vs control. Mean ± SD; n = 9, ***p* < 0.001; ****p* < 0.0001.

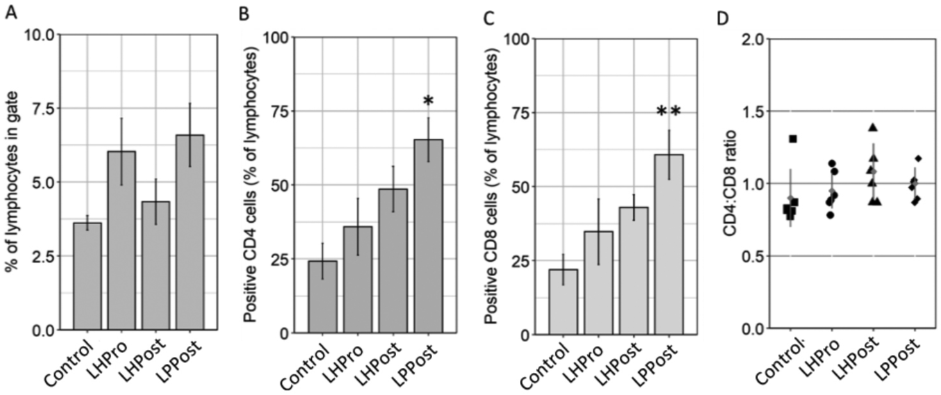

Fig 3. **A)** Average percentage of gated lymphocyte cells per treatment. **B)** Relative abundance (% of lymphocytes) of $CD4^+$ cells and **C)** CD8+ cells. **D)** Ratio of $CD4^+$ and $CD8^+$ cells in the lymphocyte population. Mean ± SEM except D) Mean ± SD; n = 6, **p* < 0.05; ***p* < 0.01.

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LOW-COST DENITRIFICATION IN BRACKISH WATER AQUACULTURE PRODUCTION

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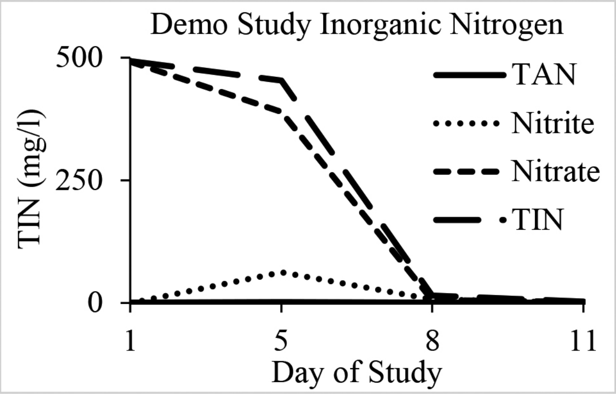
Nitrate accumulation is a primary limiting factor of water reuse in many recirculating aquaculture systems (RAS). Water reuse is critical in inland marine RAS, as expensive artificial sea salts are often used to create salt water. Denitrification, a process in which facilitative anaerobic microbes reduce nitrate to dinitrogen gas, may allow producers to reuse water for extended periods of time. This may result in significant cost savings for producers and may have positive environmental impacts by reducing wastewater discharge. This presentation will cover several past and ongoing trials investigating simple, low-cost denitrification methods in brackish water aquaculture systems.

Three studies and a demonstration project were conducted to examine different denitrification strategies. The first study examined the effectiveness of simple, external denitrification chambers containing different media types. The treatments included plastic bio-media (BM), expanded clay pellets (EC), woodchips (WC), and a control (CO) using no media. A second study examined the addition of ethanol at a 2:1 carbon: nitrogen ratio to 4 different treatments filled with water from an active shrimp production system. The treatments were aerated with ethanol, aerated with no ethanol, no aeration with ethanol, and no aeration with no ethanol (AWE, ANE, NAWE, and NANE). The third project examined denitrification in used aquaculture water using different carbon: nitrogen ratios (1:1, 3:1, 5:1, 0:1) created through ethanol additions in systems with no aeration. The demonstration project tested a denitrification method using ethanol at an active shrimp farm in a 17 m^3 shrimp tank.

The first study showed that all media types and the control (no media) can denitrify to some extent, which led to further investigations using low oxygen/low ORP environments in bodies of water without an external denitrification chamber. The second study showed a significant amount of partial denitrification in the NAWE treatment, but this treatment also had a large amount of nitrite accumulation, indicating insufficient carbon or insufficient time. The third study showed over a 95% reduction of nitrate in the 1:1 and 3:1 treatments over the course of 27 days. The demonstration study showed a significantly faster rate of denitrification, likely due to temperature. Nitrate fell from 495 mg/l to 2.9 mg/l in 11 days and only required 35 L of ethanol to denitrify 17 m^3 of water. This has important implications for shrimp producers, as a rapid and low-cost denitrification method may significantly increase the longevity of water reuse and greatly reduce water discharge and salt use.



NON-HALOPHYTE PLANT SPECIES IN BRACKISH-WATER AQUAPONICS

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Brackish-water aquaculture producers often face challenges with nitrate accumulation to the point it becomes toxic to animals. Disposing of high-nitrate water wastes both water and salt, can be toxic to terrestrial crops, and often cannot be discharged into municipal sewer systems. Using aquaponics to reduce waste discharge while producing marketable plants is gaining popularity globally; however, brackish water producers are typically thought to be limited to halophytes, or salt-tolerant plants only. Although halophytes grow well and remove nitrogen in brackish water systems, the plants are often unknown to consumers, or are not palatable. Finding non-halophyte species that are tolerant of salt and better known to consumers may allow producers to utilize aquaponics and reduce water and salt use.

Over 10 different plants have been tested at KSU to determine their suitability for brackish water aquaponics. Each plant is evaluated for salinity tolerance by growing plants in 0, 5, 10, 15, and 20 salinity. Plants that show high survival and growth at elevated salinities are used in further trials that examine plant performance in situations representative of shrimp farming conditions. Variables such as varying nutrient concentrations, plant age, acclimation strategies, decoupled versus coupled aquaponics with shrimp, and the effects of supplemental iron on chloride tolerance have been tested.

Kale has been a standout performer with 100% survival even at 20 salinity. Mustard has also shown high survival while only showing moderate growth reduction at higher salinities. A primary finding is the importance of an acclimation period. This acclimation process involves germinating plants in freshwater, then increasing salinity over a two-week period. Many plants show survival at 5, 10, and even 15 salinity when using this acclimation period. Changes in nutrient uptake and tissue concentrations have been noted, particularly replacement of Ca and K by Na, while Mg seems to be unaffected. Some plants appear to store excess Na in leaf tissue, while other plants (notably kale), appear to have lower amounts of Na in the leaves and can reduce Na uptake at the roots or have a Na transport/evacuation ability. Nitrate uptake rates tended to decrease as salinity increases across all plants, however significant amounts of nitrogen were removed from the water, even at 15 and 20 salinty. Other findings are the increased necessity of dosing certain nutrients at high levels compared to freshwater, particularly iron. This line of research has opened potential opportunities for brackish-water aquaculture producers. Further research will include human sensory profiles of the plants and consumer acceptance studies.

EXPERIMENTAL HANDLING CHALLENGES AFFECT THE MONOAMINE SYSTEM, PHAGOCYTIC CAPACITY AND TRANSCRIPTOME OF MARAENA WHITEFISH (*Coregonus maraena*)

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Introduction

Maraena whitefish *Coregonus maraena* responds to stressors in a more sensitive fashion[1] than salmonids that are better adapted to husbandry conditions, such as rainbow trout or Atlantic salmon. There are only limited reports on the physiological reactions of maraena whitefish to unavoidable aquaculture procedures. However, the response to environmental challenges is reflected on multiple physiological levels (Fig. 1a). A comprehensive panel of biomarkers provides a reliable source of information for evaluation of the actual immune status and the impact and severity of such a challenges. We analysed the monoaminergic response in the brain, the phagocytic capacity of myeloid head kidney cells and the transcriptomic response of the head kidney to acute and repeated exposure to handling of maraena whitefish.

Material & Methods

Maraena whitefish with a start weight of about 200 g were subject to a single handling challenge followed by sampling after 3 h (n = 8) and 24 h (n = 8) or repeated daily handling over a period of 10 days (n = 8); an equal number of untreated control animals were included. For the single handling challenge, a total of 16 fish were intermittently chased and netted for 1 min. They were then lifted separately from the tank and transferred into a secondary tank, where they were kept until the 3 h or 24 h post-handling time point. The repeated handling procedure was applied on a daily basis for a 10 day period, and the eight treated fish were sampled at 24 h after the last handling treatment. Cohorts of control fish (24 fish in total) were kept in parallel under identical conditions but not exposed to the handling challenge. The plasma cortisol concentrations were quantified using a competitive enzyme-linked immunosorbent assay. Microarray-based gene-expression analysis of head kidneys was performed on 8×60 K AgilentSalmon Oligo Microarrays following the Agilent 60-mer oligo microarray processing protocol. RNA isolated from brain was used for expression profiling of panel of relevant genes. A suspension of head-kidney cells was incubated with or without fluorescein isothiocyanate-labeled *Aeromonas salmonicida*. The cell suspension was then analysed by fluorescence activated cell sorting using a MoFlo XDP high-speed cell sorter.

Results

Brain neurochemistry responded to a single episode of handling with increased serotonergic activity 3 h post-challenge. This was accompanied by a modulated expression of monoaminergic receptor genes in the hindbrain (Fig.1b) and a significant increase of plasma cortisol (Fig.1c). Microarray analysis of head-kidney samples from the challenged and control fish revealed 12 and 70 differentially expressed genes at 3 h and 24 h, respectively, after the single handling episode. Only 12 genes were found differentially expressed after 10 days of repeated daily handling (Fig.1d). The identified genes were assigned to numerous stress- and immune-relevant functional pathways, including “glucocorticoid receptor signaling” (3 h post challenge), “HIF1A signaling” (24 h post challenge), or “complement system” (10 days of repeated challenge).

The phagocytic capacity of myeloid cells from maraena whitefish was significantly decreased by 37% at 3 h after handling compared with the undisturbed control fish (Fig.1e).

Discussion and conclusion

Our data indicate that relatively short handling procedures have a measurable impact on the physiology of maraena whitefish and should be minimized as much as possible to prevent increased disease susceptibility. Our data also show that habituation may decrease the activation of the stress axis and subsequent immune suppression. Therefore, close monitoring of the wellbeing of fish in aquaculture is essential to establish a balance between challenges that cause distress and subtler challenges that can lead to habituation.

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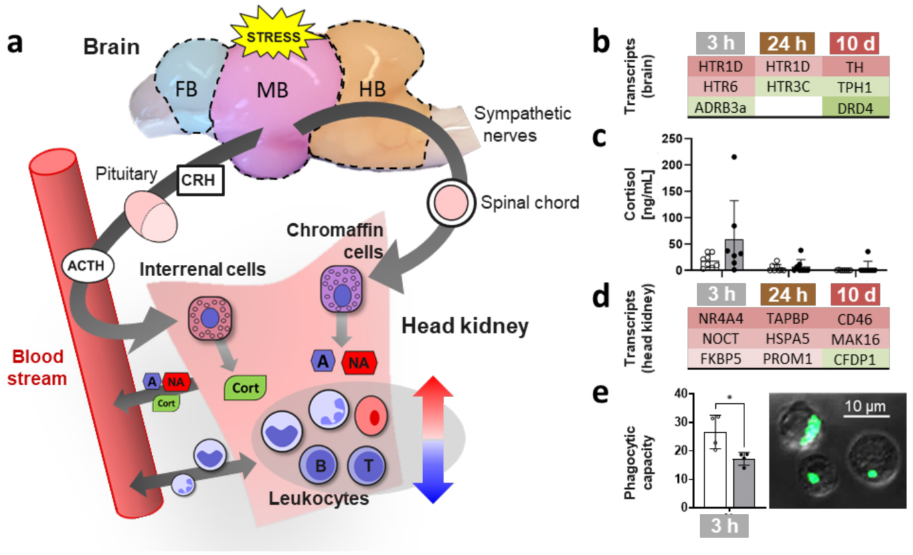

Fig.1 **Graphical summary** about (**a**) the three levels of the stress response in teleost fish and (**b-e**) major results of the present study. (**a**) The primary response activates the brain-sympathetic-chromaffin axis transferring the stress signals to chromaffin cells in the head kidney, which releases the catecholamines adrenaline (A) and noradrenaline (NA) into the bloodstream. In parallel, the activation of the hypothalamic–pituitary–inter-renal axis stimulates the release of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) into the blood. ACTH, in turn, induces the inter-renal cells in the head kidney to release cortisol (CORT) that promotes the secondary response of reorganizing the availability of energy resources. The tertiary response includes the reprograming of the immune system, behaviour, growth, and reproduction. (**b**) The most differentially expressed genes in brain 3 h and 24 h after single handling and 10 days after repeated daily handling. (**c**) Plasma cortisol concentrations in the treated (grey bars) and control (white) maraena whitefish. (**d**) The most differentially expressed genes in head kidney 3 h and 24 h after single handling and 10 days after repeated daily handling. (**e**) Phagocytic capacity of myeloid cells from treated (grey bar) and control (white) maraena whitefish 3 h after a handling challenge; confocal microscopy was used to validate the phagocytosis of labelled bacteria by myeloid cells.

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INCLUDING FAR-FIELD IMPACTS AND LOCAL CONTEXT IN THE ECO-CERTIFICATION OF MARINE SALMON FARMS

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The overall production of marine salmon aquaculture and the production of eco-certified marine salmon aquaculture have increased significantly over the past 20 years. Increasing demand for eco-certified seafood indicates that eco-certification has been successful in creating a market for sustainable seafood, but the potential for eco-certification to produce positive sustainability outcomes is less certain (Jonell et al., 2013; Tlusty, 2012; Tlusty & Thorsen, 2017). Two limitations of eco-certification in improving salmon farming sustainability are: (1) the application of global eco-certification criteria in specific and unique local contexts, and (2) the risk of excluding far-reaching impacts of aquaculture in the farm-scale application of eco-certification schemes (Amundsen et al., 2019; Amundsen & Osmundsen, 2019; Bush et al., 2019). Therefore, the goal of this study is to explicate the role of local context and the far-field impacts of aquaculture in the eco-certification of marine salmon farms.

Interviews with industry professionals who have experiential knowledge in the application, auditing, and/or design of eco-certification criteria took place over a year-long period. Conversational interviews consisted of open-ended questions about the role of eco-certification in marine salmon aquaculture. Additional materials identified through interviews and review of eco-certification scheme websites were also used to explore the role of farm location and far-field impacts of aquaculture in the application and outcomes of the eco-certification of salmon farms. Thematic coding of interview transcripts, eco-certification criteria, and variance requests were used to identify common challenges in the application of eco-certification in local contexts and how far-field impacts are considered and evaluated in the application of eco-certification criteria.

Preliminary results indicate that differences in auditor approach, training, and experience play a role in resolving conflict between local context and eco-certification criteria. Although eco-certification is a farm-scale approach to sustainability, some eco-certification schemes incorporate an ecological perspective by including criteria that reference regulatory requirements such as environmental assessment and participation in area-based management plans.

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COMPARATIVE STUDY ON EPA AND DHA CONTENT AND FATTY ACID PROFILING OF CERTAIN MARINE BY-CATCH FISH SPECIES AVAILABLE IN INDIAN WEST-COAST WATERS WITH COMMON INDIAN FOOD FISHES

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Introduction

The estimated level of capture fisheries in India is about 5.4 million tonnes. Of these 3.8 million tonnes is contributed by marine sector. The by catch landings in India is about 58000 tonnes which constitutes nearly 2% of the total landings. Among the by-catch, deep sea fishes constitute a good quantity of discards from deep sea trawlers operating at off south west coast of India. Harvesting of under-utilised fish resources is one of the effective methods to increase the capture fish production. Moreover, it also allows the recovery of over exploited conventional fish resources. Under exploited marine fishes caught are a found to be a hopeful potential resource. An attempt has been done to compare the chemical composition and fatty acids profiling of three mesopelagic fish species found in Indian west- coast waters *viz Canthidermis maculata, Gephyroberyx darwinii* and *Satyrichthys adeni* with three commonly available Indian food fishes *viz Sardinella longiceps, Rastrelliger kanagurta* and *Stolephorus commersonii*.

Materials and methods

The fish species were filleted, skinned and the homogenized fish samples were used for nutritional analyses. The samples were determined by AOAC method (2005) for moisture content, total nitrogen, crude fat an ash content. Analysis of fatty acid methyl esters of lipid extracted by the method of Folch et.al (1957) were analysed with Gas Chromatography equipped with Elite-225 capillary column (60m length and 0.25 ID) and Flame ionizing detector using nitrogen as carrier gas. Calibration of GC was done using standard FAME mixtures (Supelco Germany). Analyses were repeated three times and the results were expressed as mean ± standard deviation.

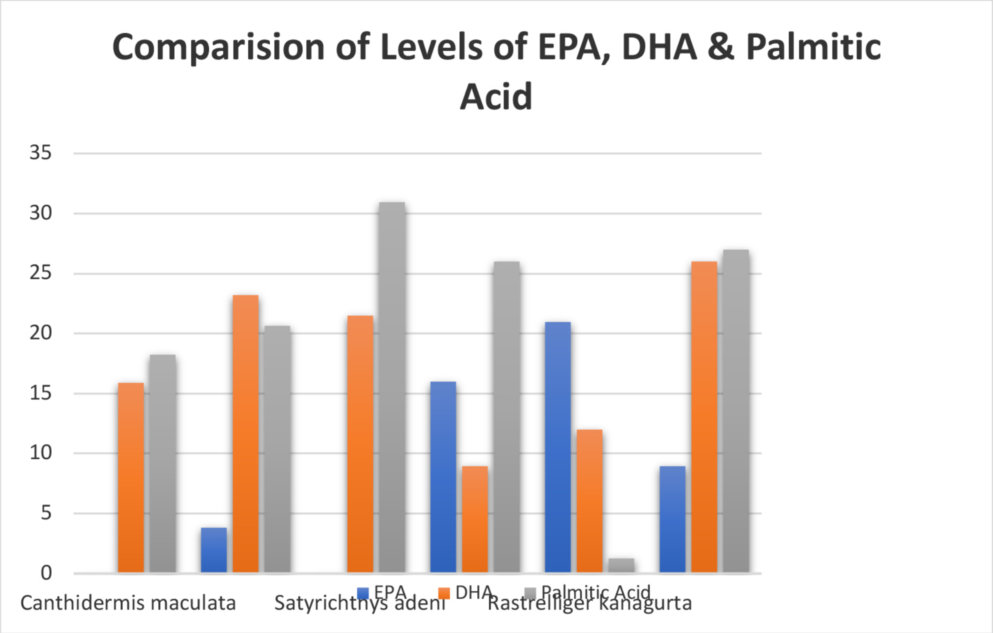

Fig 1: Comparison of levels of EPA, DHA and Palmitic Acid in *Canthidermis maculata, Gephyroberyx darwinii* and *Satyrichthys adeni* with *Sardinella longiceps, Rastrelliger kanagurta and*

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Results

Nutritional profiling has revealed a good amount of protein in all by-catch fish species *viz Canthidermis maculata* (21.99%)*, Gephyroberyx darwinii* (19.97%) and *Satyrichthys adeni* (20.99%) than the food fishes *viz Sardinella longiceps, Rastrelliger kanagurta* and *Stolephorus commersonii*. Substantial level of docosahexaenoic acid (DHA) is found in marine species viz *Canthidermis maculata* (15.94%), *Gephyroberyx darwinii* (23.23%) and *Satyrichthys adeni* (21.52%) is higher than the levels in food fishes. Furthermore, the species *Canthidermis maculata* (18.26%), *Gephyroberyx darwinii* (20.68%) and *Satyrichthys adeni* (30.98%) has considerable amount of palmitic acid as that of food fishes *Sardinella longiceps* (26%)*, Rastrelliger kanagurta* (1.24) and *Stolephorus commersonii* (27%). Of the three mesopelagic fish species, only one species *Gephyroberyx darwinii* (3.87%) showed remarkable levels of eicosapentaenoic acid (EPA). The presence of caproic acid in significant level is also evident in *Canthidermis maculata* (59.70%) and *Satyrichthys adeni* (14.64%). The results of this study show that these marine species can be considered as a potential nutrient resource in the formulation of fish-based products, pharmaceuticals and also in animal feeds.

The blue bar depicts the EPA, orange shows the level of DHA and palmitic acid in species is shown in ash bar.

GENETIC VARIABILITY WITHIN AND ACROSS SEA CUCUMBER POPULATIONS FROM DIFFERENT MEDITERRANEAN SITES

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Introduction

Sea cucumbers (class Holothuroidea, phylum Echinodermata), also named holothurians, are widespread all over the world seas and play an important ecological role for their bioturbation and alkalinization activities of the seabed. These species are also considered economically relevant for Asian luxury food (e.g. trepang) and traditional medicine markets, as well as for the production of pharmaceutical and cosmetic bioactive compounds. Their economic relevance has caused an over-exploitation of holothurians to supply these specific markets, especially in the Mediterranean area where the excessive fishing is a quite recent phenomenon. The massive withdrawal of sea cucumbers from the Mediterranean Sea (hundreds of tons of holothurians per year) has been demonstrated to have a negative impact on the marine benthonic ecosystem and caused a biodiversity loss among populations and in some places their complete extinction. For these reasons, few initiatives are recently evaluating sea cucumbers as novel aquaculture species.

The aim of this study was to evaluate the level of genetic diversity within two Mediterranean sea cucumber species, *Holothuria polii* and *Holothuria tubulosa*, across different Mediterranean areas, before considering the implementation of conservation actions and as a first step in establishing cultivated stock populations. We analyzed portions of *cox1* and *16S* mitochondrial gene sequences because of their informativeness both for species identification across the genus *Holothuria* and for genetic variability estimations within and across different populations.

Materials and methods

A total of 240 holothurians, 120 belonging to *H. polii* and 120 to *H. tubulosa*, have been sampled from three sites across the Mediterranean Sea in a range of depth from 8 to 22 meters, on different substrates seabed typologies (sand, rock, *Posidonia* meadows). Total DNA was extracted from the integument of all specimens, then amplified with specific primer pairs targeting *cox1* and *16S* genes of mtDNA of both species and Sanger sequenced. A Neighbour Joining (NJ) phylogenetic tree has been generated using 1000 bootstrap replicates from a reduced dataset of 34 *cox1* sequences, including six samples per species per site and two outgroups (*H. scabra* and *H. forskali*). Then, genetic divergence (*p-distance*) within and between species have been calculated using the Maximum Composite Likelihood model assuming the rate variation among sites with a Gamma distribution (shape parameter = 1).

Results and discussion

The estimation of genetic diversity shows that the average *p-distance* is similar between and within species (0.10 and 0.09, respectively; Table 1).

Figure 1 shows the phylogenetic tree derived from of the holothurian mtDNA *cox1* gene. A clear differentiation between the two species is evident, confirming the informativeness of this mtDNA gene region, which can be also applied also for authentication purposes of sea cucumber derived food products. Moreover, the NJ tree shows no differentiation within species between site populations, indicating the existence of active gene flows among Mediterranean sites populations.

Finally, evidence of heteroplasmy in mtDNA sequences of a total of five holothurians of both species have been identified, suggesting that some hybridization events might have occurred between these two sympatric species, as already reported in other studies. Figure 1 shows two of the five animals in which heteroplasmy was detected: their position in the tree is nested to the cluster of their respective species.

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Genetic diversity	Average	Standard Error
Inter species diversity (p-distance)	0.10	0.03
Intra species diversity (p-distance)	0.09	0.02

Table 1. Genetic diversity (p-distance) between and within *Holothuria polii* and *Holothuria tubulosa.*

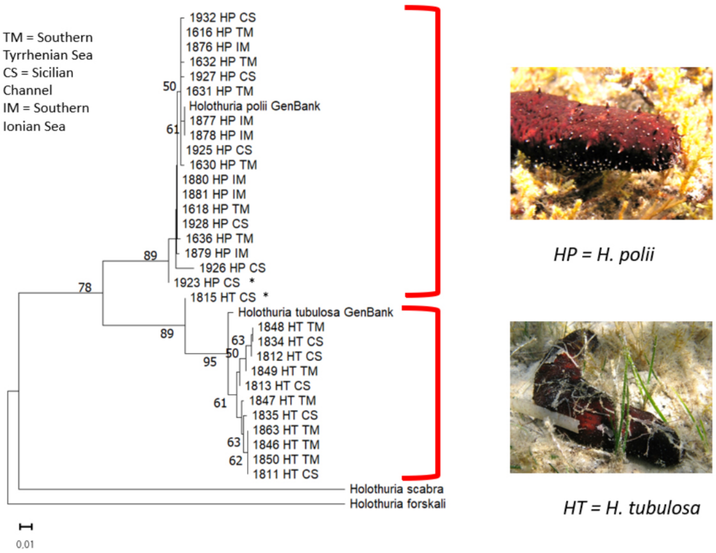

Figure 1. Neighbour-Joining phylogenetic tree of three populations of *H. polii* (HP) and *H. tubulosa* (HT) in Mediterranean Sea (TM = Southern Tyrrhenian: CS = Sicilian Channel; IM = Southern Ionian). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. * Samples in which evidence of heteroplasmy have been identified. For these sequences, the possible two mtDNA haplotypes were not inferred.

Conclusions

The results of this study provide genetic markers useful for holothurian species identification and an overview of the genetic variability existing in three populations of *H. polii* and *H. tubulosa* in the Mediterranean Sea. Further studies are needed to disentangle the origin of the mtDNA heteroplasmy reported in a few samples. These data will be useful to establish cultivated stocks with high level of genetic variability.

Acknoledgements

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WILL CONSUMERS BUY FISH FORTIFIED IN OMEGA-3?

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Introduction

Fish and aquatic foods are associated with numerous health benefits not just as a source of protein, but also as an exceptional and diverse provider of essential omega-3 fatty acids and bioavailable micronutrients. Aquaculture is the fastest growing agri-food sector globally and has huge potential to feed and nurture the world's growing population (FAO 2022). However, aquaculture of carnivorous fish is highly dependent on fishmeal (FP) and fish oil (OP) from wild fish stocks, creating an urgent sustainability challenge. The partial replacement of FP and OP by plant ingredients is already a common practice in the industry. However, concerns arise about the effects of FP and OP substitutions on the nutritional value of fish muscle and on the content of concomitant beneficial nutrients for humans, as plant ingredients used instead are devoid of the aforementioned n-3 LC-PUFA. Since one of the goals of aquaculture is to promote health guaranteeing the supply of omega-3s to the consumer, recommended by the World Health Organization, it is essential to investigate strategies to overcome this problem. That is what OmegaPeixe project aims at: the production of differentiated fish with a high content of omega-3 LC-PUFA, focusing on species of great relevance and value in Southern Europe - European seabass and turbot. In order to have real impact, this innovative product must be viable on the market, meaning that the increase in costs per kg of fish produced should be lower to the price increase that the consumers are willing to pay for a high-quality product, rich in omega-3s. A comprehensive consumer survey was carried out nationally and in the relevant European countries to evaluate the market's acceptance of differentiated aquaculture products, considering the general consumer segment and also the Organic products sector.

Objectives

To carry out a comprehensive consumer survey, both nationally and in relevant European countries, a questionnaire was developed with the main purposes of:

1. Assess the consumer's willingness to pay for omega-3 fortified aquaculture fish.
2. Assess the level of consumer confidence in aquaculture products.
3. Identify trends in fish consumption.

Material and methods

The online questionnaire was designed and adjusted, considering the multivariate analysis carried out on the pre-test to allow for the collection of information on the target audience - socio-economic characterization data, consumption data of fish and aquaculture products, willingness to pay for value added products, level of trust and preference for aquaculture products. The questionnaire was disseminated by the project promoters, through their commercial and institutional contact networks, as well as through social networks. Additionally, relevant consumer entities from the aquaculture sector and NGOs were approached to participate in the dissemination of the questionnaire to make it as comprehensive as possible. Responses were collected over a 5-month period from September 16, 2021 to February 16, 2022. The information collected was statistically treated in IBM SPSS Statistics 28.0 program applying Multivariate Analysis so that hypotheses and relationships between variables could be assessed.

Results

In this study 1349 valid questionnaires were obtained, with respondents aged 18-82 years, 61.7% living in Portugal and 30.9% living in Spain. 75.7% of the respondents live less than 20 km from the coast and 84% in urban areas; 80% are active, the average monthly household income is over 3000 € for 21.1% of the respondents and 33.4% spend more than 400 € monthly on food. Additionally, 88.3% have never worked in the aquaculture sector and 97.4% are fish consumers. The most reported consumed fish is salmon (60.9%, 800 responses), followed by cod (52.2%, 689) and tuna, with similar values of preference (51.7%, 680). 35% consume sea bass and 2.6% consume turbot. 45% of the fish consumers prefer fish of national origin, with only 2.5% preferring fish from outside the European Union. The respondents are well informed, as 88.9% know what omega-3s are. About 50% of fish consumers and more than 45% of those who do not usually consume fish would be willing to pay at least 1€/kg more for fish fortified in omega-3. 37.1% of respondents that do not consume fish would consider eating it if they knew that it was an enriched food and that it provided an extra health benefit.

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Acknowledgements
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HISTOPATHOLOGICAL EFFECT OF *Microcotyle sp.* (MONOGENEA: POLYOPISTHOCOTYLEA) ON GILLS OF *Diplodus cervinus* PRODUCED IN AQUACULTURE

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Introduction

The monogenea parasites are responsible for the majority of stock losses, leading to economic losses in aquaculture, affecting several species (Doan et al, 2020). In Mediterranean aquaculture, sea breams are also produced, as are *Sparus aurata* and *Dicentrarchus labrax*. This is the case of *Diplodus cervinus*, which is produced in land-based tanks, notably in polyculture with other species. Most bream are more sensitive to ectoparasites than other commonly produced species such as sea bream, sea bass and meagre. The aim of this paper is to describe how the monogenea *Microcotyle* spp. affects the gills of *D. cervinus* and to analyze the level of infection.

Methods

An anomalous behavior of reared fish was observed in a earthen pond of a semi-intensive production system at the Aquaculture Research Station of Olhão. Thus, six *D. cervinus*, were collected for pathological analysis, microbiology, external and internal parasites observation. Firstly, the animals were sacrificed with a spin column insition and the four left-sided gill arches were removed and the presence of parasites was verified and counted under an optical microscope. After that, the first two gill arches on the right side were collected for histology, placing them in 4% buffer formalin for 48 hours and transferred to 70% ethanol. The slides were sectioned and stained with haematoxylin and eosin. Finally, the mounted slides were scanned with Hamamatsu NanoZoomer C13140-01 and the images visualized with the NDP. The level of lesions was classified according Poleksic & Mitrovic-Tutundzic (1994).

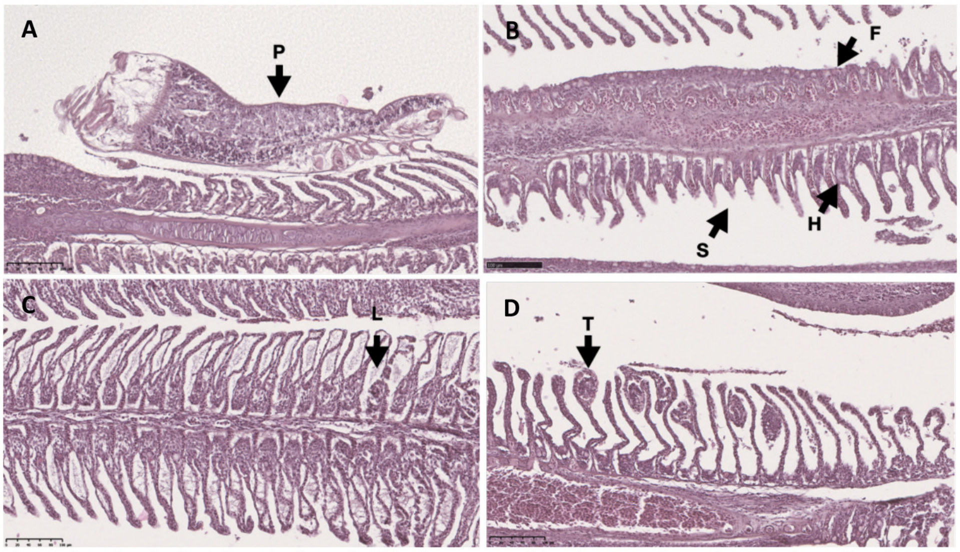

Figure 1 - Histological description of gill lesions caused by the parasite *Microcotyle sp.* in *Diplodus cervinus*, A) P - part of the parasite *Microcotyle sp.*; B) F - fusion; H - hyperplasia; S - shortening of the secondary lamella; C) L - lifting of filaments; D) T - lamellar telangiectasis

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Results and Discussion

Water temperature in ponds was 19,5 ± 1°C. *D. cervinus* exhibited swimming near the surface. Thus, a pathological analysis was performed revealing the presence of monogenean *Microcotyle sp.* in the gills and its mean intensity was 1,83 (1-6) parasites per gill arch in each fish. Concerning other pathogens, there was growth of *Pseudomonas* sp. on plate . However, no other external and internal parasites were observed in the remaining fish.

With the presence of the parasite, observed in histological sections, the gills presented some alterations, the most recurrent were hypertrophy and hyperplasia of gill epithelium, lifting of epithelium cells, rupture lamellar, fusion and shortening of secondary lamellae, filament blood vessels enlargement, thinning or thickening of respiratory epithelium, lamellar telangiectasis and in some places degradation of the epithelium may occur (Figure 1). In general, the severity of lesions was classified as moderately to heavily, However, many of these changes belong to the first phase, in which there is not a high severity in the tissue, managing, through appropriate environmental conditions, to recover the normal structure of the tissue.

In conclusion, the monogenea *Microcotyle sp*. causes some lesions on the gills of the host *D. cervinus,* and it is necessary to implement prevention and mitigation measures for this parasite, so that infections decrease in aquaculture.

Acknowledgments

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NOVEL METHOD FOR MICRO AND NANOPLASTICS DETECTION, COUNTING AND CHARACTERIZATION IN RAW INGREDIENTS FOR AQUAFEEDS

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Introduction

Plastics are ubiquitous pollutants found in marine, freshwater, and terrestrial environments. Microplastics (MPs) are defined as plastic particles ranging from 1μm to 1 mm in diameter, whereas nanoplastics (NPs) have a particles size range of 1-1000 nm. One of the main risks deriving from MPs and their nanoscale forms is their bioaccumulation along the food chain, which potentially represents a threat not only for the environment but also for human health. There are evidences of MPs accumulation in the digestive system of fish causing various adverse effects, such as damage to the gastrointestinal tract, cytotoxicity, and dysbiosis (Montero et al., 2022). The translocation of MPs from feed to fillet has been also detected in two of the most important commercial fish species: gilthead sea bream (*Sparus aurata*) (Jovanović et al., 2018), and European sea bass (*Dicentrarchus labrax*) (Zeytin et al., 2020). As a consequence, humans may be exposed to high MP and NP levels by seafood consuming.

The MPs and NPs identification are becoming an emerging issue for consumer health, and recently a new analytical method of identification in complex matrices based on *μ*-Raman spectroscopy has been developed (Valsesia et al., 2021). The present research represents one of the first attempts to evaluate the presence of MPs and NPs in a commercial raw material used for aquafeed formulations, such as meal from black soldier fly (*Hermetia illucens*) larvae, which is considered a valid and sustainable alternative to fishmeal. Insects for aquafeeds are generally produced in a frame of circular economy, as agri-food wastes are commonly used as rearing substrate for their larvae.

Materials and Methods

Sample preparation. An aliquot of commercial insect meal was digested overnight with 10% (wt/vol) KOH at 60°C. Two sequential vacuum filtration steps of digested sample were performed on polycarbonate filter membranes (12 μm) and then, on a pore size filter of 2 μm.

Confocal Raman microscopy (CRM) analysis of 2-12 μm plastic fraction. Analysis of filter was performed using an inVia™ Confocal Raman microscope (Renishaw). Optimization of Raman signal was set as follows: 1 s, 10% laser, 10 accumulations, range 1700-3000 cm^{-1}. Extended analysis of spectrum (200-3500 cm^{-1}) was performed on more interesting objects setting the Raman signal at 5 s, 10% laser, 5 accumulations.

Scanning electron microscopy (SEM) analysis of < 12 μm and < 2 μm plastic fractions. An aliquot of digested sample was filtrated on 12 μm pore size polycarbonate filter membrane and the eluate was manually spotted on a silicon (Si) Teflon-coated wafer and dried under nitrogen flux before imaging. To analyze plastic particles of less than 2 μm, filtrate from 2 μm pore size filter was firstly concentrated using an Amicon Ultra 2 mL Centrifugal Filter Unit (30,000 MWCO) and then, manually spotted over a rough superhydrophobic surface (Si wafer coated with polydimethylsiloxane). The presence and distribution of small plastic particles was performed by spotting sample on a positively charged surface to immobilize the particles in random separated positions thus permitting SEM counting and Raman identification. Scanning Electron Microscopy of samples was performed using a Nova 600i Nanolab equipped with an Energy Dispersive X-Ray spectroscopy (EDS) system for elemental analysis.

Transmission Electron microscopy (TEM) Analysis of < 2 μm plastic fraction: TEM coupled with Energy Dispersive X-Ray spectroscopy (EDX) was used at 120 kV in both TEM and STEM modes to characterize primary size, morphology and elemental composition of plastic-like particles, in the nanosize range dimension. The elemental analysis was done in STEM, bright field, and hypermap mode (Quantax software, Brüker, Italy) to identify the carbon content.

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Results

The KOH treatment efficiently digested the organic matter of lipid-rich insect meal sample eliminating a large part of the biological matrix. Six different areas of filter corresponding to 0.01% of the whole filtering surface were analyzed with Renishaw CRM. A total of 328 objects were manually recognized and mapped. Among them, seven were recognized as polymers of polyethylene (PE) with a size < 10 µm. Considering the volume of sample filtered and the fraction of area of the filter analyzed at best resolution, the potential hit of polymer objects in the whole sample was calculated. In this case we found a concentration of 7 x 10^5 particles in 0.5 g of insect meal.

SEM counting and Raman identification performed on sample (< 2µm fraction) spotted on positively charge surface confirmed the presence of nanoparticles of PE in insect meal. Accordingly, the same analysis performed on sample spotted on superhydrophobic silica surface established the presence of aggregates of PE particles.

TEM coupled with EDX confirmed the presence of plastic-like morphology structures inside the digested insect meal sample. The corresponding EDX analysis in STEM mode displayed that these objects contained carbon in higher concentration consistent with PE plastics.

Conclusions

In conclusion, our results demonstrated the presence of MPs and NPs in commercial insect meal. The method used was suitable even for the extraction of nano size particles. A combination of Raman microscopy, SEM/EDS, TEM/EDX led to a successful detection of both micro and nanoplastics in a complex organic matrix such as insect meal with a reliable estimation of their concentration.

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EFFICIENCY OF A NEW RAS IN THE REMOVAL OF MICROPLASTICS

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Introduction

Plastics are a ubiquitous source of marine pollution, especially in the form of microplastics (MP). Exposure and contamination of fish and other seafood products by MP is a major concern worldwide and brings two types of risks to fish culture: 1) disruption of fish growth and reproduction thereby impacting operational capacity and 2) possible adverse health effects on consumers of fish products contaminated by MP. Culturing of fish in onshore recirculating aquaculture systems (RAS) can on the one hand potentially reduce exogenous MP contamination, i.e., from seawater, via water filtration, but on the other hand may expose fish to endogenously generated MP, i.e., from within the RAS plastic components, or even be contaminated through fish feeds derived from marine sources. Aiming for two major objectives: 1) knowing whether MP contamination in RAS-cultured fish is higher, lower, or equivalent to fish grown in open sea cages or wild-captured fish, and 2) knowing if MP characteristics (particle size and chemical composition, including plasticisers and other additives) are different for RAS-grown fish in comparison to fish obtained from other sources; a consortium formed by SEAentia (RAS meagre producer) and 2 research institutes, IPMA and SINTEF, implemented the project "Minimizing microplastic contamination in onshore (RAS) meagre farming" – MP-RAS, funded by EEA Grants. The aim of this study was to investigate if the RAS water contains differences in the quantity and characteristics of MP content when compared to raw seawater used to fill up the system.

Materials and methods

Over a course of four months, water samples were collected at four sampling points along a newly loaded RAS, used for the growth of meagre (*Argyrosomus regius*). The system has 2 distinct filtration systems: 1) intake water before the recirculation and 2) RAS water inside the recirculation loop. The four sampling points include the seawater inlet, collected from the seashore (SP1), after the intake water filtration and before filling the RAS with water (SP2); Inside the RAS loop, water coming from fish tanks and before entering the filtration system (SP3); and after exiting the filtration components of the recirculation and before re-entering the fish tanks (SP4). Water samples of 10 litres were collected in MP-free glass containers and filtered through 1.2-micron glass-fibre filters. The glass-fibre filters were treated with filtered hydrogen peroxide (30% solution H_2O_2) as an oxidizing agent to digest organic matter. The resulting solutions were filtrated using 20 µm stainless-steel filters and washed repeatedly with ultrapure water. These filters were then put into concentrated NaCl solutions for density separation. To detach the sediments from the filters, sonication was used for 10 min. After 10 min centrifugation at 3000 rpm, the upper part of the solutions was decanted. Lastly, both the upper and lower parts were filtrated using a Whatman Anodisc25 filter. Three successive extractions of the stainless-steel filters were carried out. The glass-fibre and Anodisc25 filters were analysed by optical microscopy using an Olympus DSX100 digital microscope. The amount of particulate matter including MP and microfibres (MF) on the Anodisc25 filters was estimated by image analysis using a Matlab script developed in-house. FT-IR microscopy (Agilent Cary 620/670) was used to assess the material type of some representative MP on the Anodisc25 filters.

Results and Discussion

The seawater used to supply the RAS contained large amounts of particulate matter including MP and MF (Figure 1). These amounts varied over time due to changes in water currents and weather events. The amount of MP and MF was reduced after the first filtration (SP2) but increased again at SP3. Since the fish tanks have no cover on top, particles and fibres from the facility's environment can enter the RAS, which might contribute to the observed increase. The filtration after the fish tanks (SP4) did not reduce the level of MP and MF significantly. A reason might be that this filter system is mainly designed to remove fish and feed-related biological waste but not necessarily MP and MF.

FT-IR analysis of the Anodisc25 filters revealed that the material types of the MP included the most common plastics, such as polyethylene, polypropylene, polyester, and polyamide (Figure 2). Whether some of these MP can be associated with certain components within the RAS is still under assessment. Many of the MF were natural fibres, such as cotton, which indicates that they most likely originate from the facility's environment.

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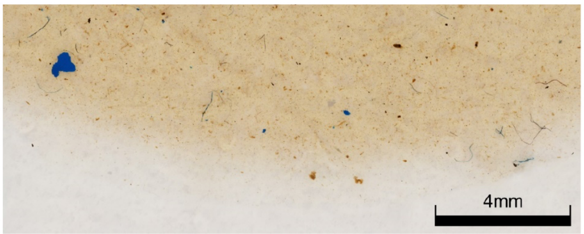

Figure 1 - Microscopy image of a glass-fibre filter from the seawater inlet (SP1).

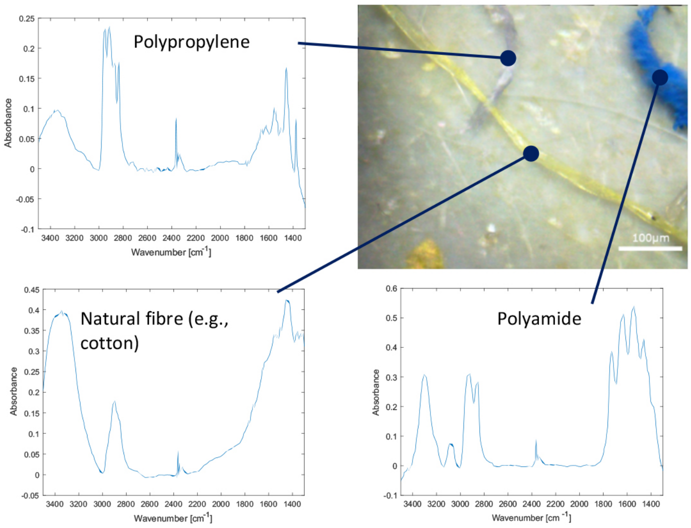

Figure 2 - Microscopy image of an Anodisc25 filter from SP3 showing MP and MF as well as FT-IR spectra of the respective items.

Conclusion

Water samples were collected at four sampling points along a new RAS. These samples were filtered and characterised by optical and FT-IR microscopy, which identified MP and MF from possibly endogenous and exogenous sources. The filtration system at the inlet of the RAS seems to significantly reduce the amount of particulate matter whereas the filtration after the fish tanks is less effective in removing MP and MF.

Acknowledgements

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MINIMIZING MICROPLASTIC CONTAMINATION IN ONSHORE (RAS) MEAGRE FARMING

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Introduction

Microplastics (MP) in the marine ecosystem are relentlessly growing, becoming a threat to food safety. This contamination will increase over the foreseeable future with MP already present in the major finfish species harvested for food (2017, FAO Technical paper 615). In aquaculture, fish exposure to MP can: 1) impair growth and reproduction, thereby affecting operational capacity, and 2) cause adverse health effects on consumers of fish products contaminated by MP. Culturing of fish in recirculating aquaculture systems (RAS) may diminish MP contamination through ultra-filtration of the water used to stock the system. However, the system might expose fish to endogenously generated MP, or through fish feed derived from marine sources. To determine the efficacy of RAS in minimizing MP levels of cultured fish, a consortium formed by SEAentia (RAS meagre producer) and 2 research institutes, IPMA and SINTEF, implemented the project "Minimizing microplastic contamination in onshore (RAS) meagre farming" – MP-RAS, funded by EEA Grants. The project will assess the efficacy of the RAS water conditioning system in MP clearance and will compare MP levels of meagre cultured in this system with meagre from wild-capture and cultured in open-sea cages. Detailed knowledge of MP levels and composition in farmed fish coupled with the deployment of technological solutions for minimizing MP contamination will provide added value to the fish product and will have a wider societal benefit by raising awareness of MP pollution while stimulating more widespread use of MP mitigation strategies among fish producers.

Materials and Methods

Analysis of water-borne MP in the RAS

Water samples were collected at four sampling points of SEAentia's pilot RAS. The system has 2 distinct filtration systems: 1) intake water before the recirculation and 2) RAS water inside the recirculation loop. The sampling points were immediately before and after of each of the filtration system. Water samples, after being filtered and processed, were analysed by optical microscopy to assess quantities of particulate matter and also by FT-IT microscopy to determine the material type of MP.

Comparison of MP and related contaminant levels in tissues of RAS-cultured meagre *versus* meagre harvested from open sea cages and by wild-capture

Fish were collected at the beginning and after 12 months of growth in SEAentia, representing RAS-culture meagre population. This approach enables the evaluation of MP content in fish tissues through time and its comparison with wild and sea cage-produced individuals. All the MP obtained from the samples were quantified by colour and shape, and the plastic polymer identified using a Fourier Transform Infrared spectrometer coupled with a universal attenuated total reflectance accessory (ATR-FTIR). MP are usually associated to other contaminants, such as organic pollutants, toxic metals, and plasticizers. The determination of these contaminants was performed applying a specific protocol optimized for this species. With all this information it will be possible to conduct a modelling study to identify the main drivers of the different MP, as well as the correlations between the contaminant content and MP and the nature and strength of such correlations.

Results and Discussion

Results will be presented and discussed with detail, when all the analyses and data treatment are completed.

Acknowledgement

We thank EEA Grants Portugal for financially support this project.

AMPLICON SEQUENCING REVEALS DIFFERENT NITRIFYING BACTERIA IN RAS BIOFILTERS WITH DIFFERENT SALINITIES

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Recirculating aquaculture system (RAS) operating at a high degree of water recirculation depends on an array of water purification technologies to maintain good water quality, including an active biofilter with high capacity of microbial nitrification for ammonia removal. However, detailed knowledge on microbial colonization, development and influencing factors on these bacteria is still deficient today, despite the use of biofilter technology in aquaculture for decades.

At Marineholmen RASLab, research and development on aquaculture species and technologies are conducted in small-scale RAS units. These units are equipped with 1000L tanks and have microbial biofilters adapted to either fresh water (0ppt NaCl), brackish water (15ppt NaCl) or sea water (34ppt NaCl). The biofilters were inoculated with commercial enrichment culture in May 2020, and have developed to specific biofilters for the 3 different salinities (0ppt, 15ppt or 34ppt NaCl) over the last 2 years. In order to obtain detailed information about the nitrifying bacteria in biofilms from all 3 types of biofilter, targeted 16S rRNA genes from the entre microbial communities were amplified and sequenced using Illumina sequencing technology.

The results revealed great differences in microbial communities present in the specific types of biofilter. Overall, the diversity in the biofilter for brackish water was much lower compared to the biofilters for fresh water and sea water. In addition, the number of common bacterial taxa, i.e. bacteria present in all 3 biofilter types, was only 64, while the number of unique bacterial taxa were 238-335. Within the nitrifying community, brackish water biofilters at Marineholmen RASLab were dominated by the ammonia oxidizing *Nitrosomonas* and nitrite oxidizing *Nitrospira*. In contrast, the fresh water biofilters were dominated by the ammonia oxidizing *Nitrosospira,* which was also present at high relative abundance in the sea water biofilter. Furthermore, the fresh water and sea water biofilters had lower relative abundances of *Nitrospira* compared to the brackish water biofilter, which was also represented by a different species in the latter biofilter type.

In all biofilters, bacteria involved in both steps of nitrification were detected, but different species of nitrifying bacteria were associated with the process in each biofilter. Hence, different salinities seemed to enrich different nitrifying species. These had different growth requirements or responses to changing conditions, but still served a similar function in the biofilters. This clearly indicates that different RAS can contain different biofilters, depending on type and management, which could make it difficult to have standardized protocols on how to operate an RAS biofilter and which response to expect from changing conditions.

TOTAL FISH OIL SUBSTITUTION BY DATE PALM SEED OIL IN *Liza aurata* LARVAE

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Introduction

Aquaculture has become a fundamental tool to meet the growing demand for seafood, to serve an increasingly demanding consumer in the variety and quality of their food. While diversification of aquaculture production is not a new issue (Abellán & Basurco, 1999; Espinós *et al*., 2011), it is accompanied by the need to supply differentiated, economically profitable, and sustainable products, and in sufficient quantity to meet a global demand that is growing exponentially (FAO, 2022). On the other hand, the search for alternative and scalable raw materials, bioresources or co-products of vegetable origin for feed, to reduce conventional raw materials, primarily meals and oils of marine origin, is essential. This is generally accompanied by difficulties in meeting the requirements of marine fish(Tocher, 2015).

Material and Methods

Four experimental diets with 0%, 50%, 75% and 100 % substitution of marine oils with *Phoenix dactylifera* seed oil were formulated and tested in *Liza aurata* larvae (29 dph) for 25 days.

Afterthe feeding trial,proximal composition, growth, survival, stress resistance and gene expression of Fads2 (fatty acid desaturase) and Elov5 (fatty acid elongase) were evaluated.

Results and Discussion

The results found in the larvae during the 25 days of feeding showed no negative effects on growth, survival and resistance to stress up to 100% substitution of marine oils. Regarding the fatty acid profile in larvae, a decrease in EPA (20:5n-3), ARA (20:4n-6) and DHA (22:6n-3) was found, related to the increase of date seed oil in the diets. On the other hand, gene expression results of Elov5 and Fads2, showed promising trends in larvae fed with the microdiets containing date seed oil, although the values were not sufficient to improve the fatty acid profile.

Therefore, larvae at 54 dph did not show essential fatty acid biosynthesis capacity to the extent reported for the same species in its juvenile stage (Quirós-Pozo *et al*., 2021). Being the first results in this line reported for *Liza aurata*, they open the door to new studies covering the development of the animal between 54 dph and its juvenile stage. Determining the evolution of the ability to biosynthesize long-chain fatty acids contributes to the development of specific feeds for mugilids throughout their culture cycle and the expected overall sustainability of commercial-scale production of these low trophic level species.

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SALMOSIM®-DIGEST, AN *IN VITRO* SALMOSIM® ASSAY FOR PREDICTING DIGESTIBILITY AND ABSORPTION IN THE ATLANTIC SALMON GUT

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Introduction

The growth of the aquaculture industry and high cost of fishmeal and fish oil has resulted in a shift within the feed sector to utilise alternative protein and lipid sources[1]. However, gastrointestinal health must be maintained and/or improved to ensure growth and robust immune responses against disease outbreaks. Currently, *in vivo* testing is the best means of determining digestibility and assessing feed quality. However, *in vivo* testing is both time-consuming and expensive, prohibitively so for many smaller producers[1,2]. *In vitro* digestibility methods have potential as a rapid and reliable way to calculate nutritional values and rank ingredients regarding suitability for inclusion in aquafeeds[2]. Recently, we have adapted the *in vitro* gastrointestinal simulator of cultivated Atlantic salmon known as SalmoSim®[3,4] to assay protein digestibility and absorption to compare against *in vivo* methodology. Here, we aim to test how well SalmoSim®-Digest predicts matching *in vivo* digestibility trial data.

Method and Materials

SalmoSim-Digest consists of bioreactors where pH and temperature parameters are maintained to simulate two different Atlantic salmon's gut compartments (first stomach, then pyloric caecum). At each compartment phase, appropriate enzymes cocktails and bile salts are added to simulate the digestion of proteinaceous aquafeed ingredients over 90 hours. Dialysis was conducted over the same period to allow the transfer of amino acids across a semipermeable membrane. Samples of dialysate were taken at 4-time points (18, 42, 66 & 90 hours) in triplicate for each bioreactor.

One millilitre of dialysate (in triplicate) was analysed for amino acid concentration by Phthalaldehyde amino acid assay with absorbance read at 340 nm with Multiskan Spectrum Spectrophotometer. Data were corrected using the absorbance of seawater (pH7) to calculate zeroed amino acid concentration. Raw ingredients were also analysed using an industry-standard pepsin assay. Apparent digestibility coefficients (ADCs) were calculated by comparing crude protein concentrations (Kjeldahl method) of undigested vs digested feed for both *in vivo* and *in vitro* assays.

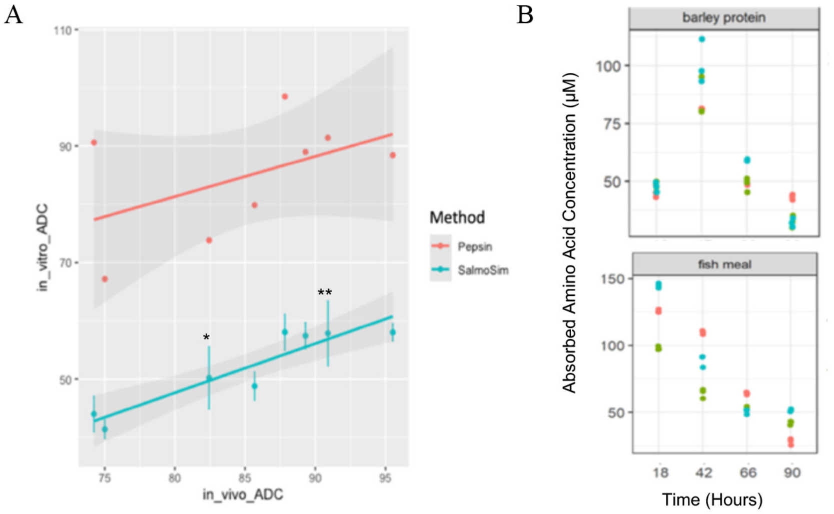

Figure 1: (A) SalmoSim-Digest comparison with *in vitro* trial data by Alltech-Coppens Ltd for *Oncorhynchus mykiss* (Rainbow trout). SalmoSim-Digest, $R^2 = 0.86$ (Blue). Pepsin digestibility assay, $R^2 = 0.23$ (Red). Based on eight feed ingredients (Barley protein * and Fishmeal **) including four different single-cell proteins. (B) SalmoSim-Digest amino acid absorption (µM) for two protein ingredients (Barley Protein and Fish meal) over 90 hours (colours indicate replicates).

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Results

The use of SalmoSim in assessing *in vitro* against *in vivo* digestibility revealed that SalmoSim-Digest can accurately predict *in vivo* digestibility trial data in salmonids ($R^2 = 0.86$), compared to ($R^2 = 0.23$) for industry-standard pepsin digestibility assays (Fig. 2A). Concentration of absorbed amino acids accumulated in the dialysate could also be reproducibly estimated at desired time points during digestion revealing distinct temporal patterns of absorbance (Fig 2B) that correspond with ADC profiles of distinct ingredients.

Discussion

We assessed the accuracy of SalmoSim-Digest in predicting *in vivo* salmonid protein digestibility and absorption. Our analysis revealed that we can accurately predict *in vivo* digestibility for proteins in salmonids *in vitro*. In this respect, our assay outperforms the current *in vitro* industry-standard pepsin digestibility assay. Alone, SalmoSim-Digest represents a rapid, cost-effective and ethical tool to assay ingredient digestibility, avoiding *in vivo* trials. Combined, SalmoSim-Digest (digestibility) and SalmoSim (gut microbial communities) offer a powerful tool to explore the link between gut microbes and ingredient digestibility in the Atlantic Salmon gut.

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LYSOPHOSPHOSLIPID SUPPLEMENTATION IMPROVES FEED INTAKE AND GROWTH OF TILAPIA (*Oreochromis sps.)* REARED AT LOW TEMPERATURE

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Tilapia was the third most farmed fish species globally in 2018 according to FAO 2020 report. Although the species has an optimum temperature range of 26-30ºC, tilapia is cultured at suboptimal temperatures in many countries around the world. The negative impacts of this practice are reflected in feed intake, growth and at last, in an impaired organ and immune system functioning. At low temperatures, several fish species go under the so called homeoviscous adaptation, which is the process to increase the fluidity and permeability of the cell membranes by increasing the fatty acid unsaturation of their phospholipids.

In the last years, lysophospholipids are coming to the scene of aquafeed formulations since they play an important role as emulsifying agent and improve the absorption of lipids and lipid-soluble compounds among other metabolic actions.

In the present work, tilapia fingerlings with an average individual weight 42,02 ± 0,34 g were stocked in 24 tanks of a freshwater recirculating aquaculture system (RAS) during11 weeks at 18ºC temperature (10ºC below optimal temperature for the species). The trial was divided in two periods with different feeding management (7 weeks automatic feeding and 4 weeks manual feeding). Lysophospholipids (Aqualyso, Adisseo) were included in the diet at different levels of inclusion.

Lysophospholipid incorporation in the feed had a positive effect on feed intake (Figure 1), feed conversion and growth of tilapia grown at 18ºC. Somatic indexes were also positively affected by the use of lysophospholipids. Results also showed a relevant role of feeding management on the tilapia culture performance.

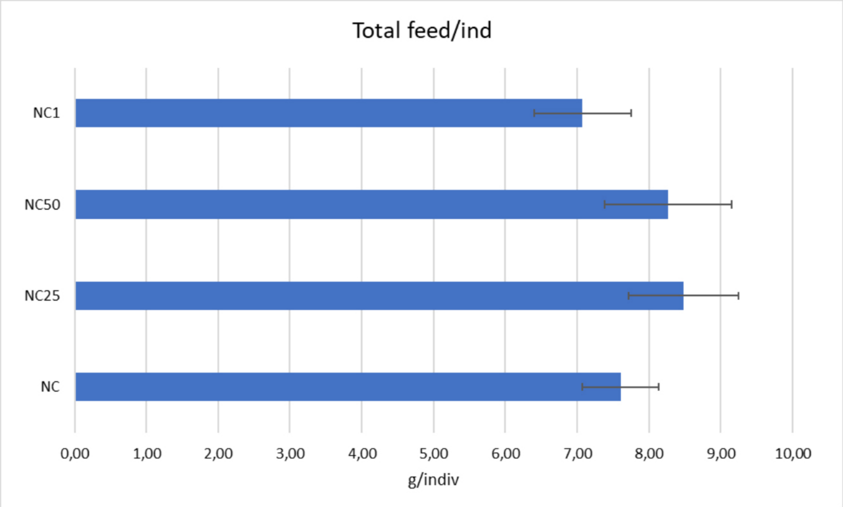

Figure 1. Feed intake, expressed as g/individual, of tilapia fed the four experimental diets (NC, NC25, NC50 and NC1).

UNDERSTANDING OFF-FLAVOUR IN WATER AND FISH OF RECIRCULATING AQUACULTURE SYSTEMS: FROM THE ECOLOGICAL SIGNIFICANCE OF GEOSMIN TO FLAVOR DECRYPTION THROUGH TRADITIONAL AND NOVEL ANALYTICAL APPROACHES

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Introduction

Recirculating Aquaculture Systems (RAS) allow for raising fish in a land-based and controlled environment to minimize the direct interactions between production processes and the environment (Ahmed & Turchini, 2021). Even though RAS farms can produce high-quality fish, "musty" and "earthy" off-flavours often taint fish, which can cause significant financial losses for fish farmers (Podduturi et al., 2021). The main volatile compounds that impart those flavours in fish are geosmin and 2-methylisoborneol, besides many other compounds that may additionally play a role in fish off-flavour (Moretto et al., 2022). Traditional procedures to deal with these problems in RAS include oxidation processes, such as ozonation, or fish depuration in cleaner water. The last approach causes weight loss and fish stress, and is also energy- and water-demanding (Podduturi et al., 2021). Scientific and industrial efforts have been applied to minimize these problems, and it has been considered of great importance to use accurate tools for early detection of the presence of microorganisms producing off-flavours, such as highly sensitive molecular biology and advanced sensory analytical techniques to avoid expenses associated with the purification process (Moretto et al., 2022). In a newly established European network project (RASOPTA), novel instrumental and microbiological approaches are combined to help alleviate the present off-flavour issues in RAS production of commonly farmed fish within the EU.

Methods

A workflow developed by four PhD students aiming to comprehend odour issues in RAS is presented. It covers geosmin production ecology and degradation dynamics, influence of fish feed, sensory analytical characterization and quantification of odour-active compounds and consumer acceptance. Specifically, the present work will apply microbiological assessments, such as genetic engineering to study the regulation of the geosmin synthase gene, and bioinformatic tools to identify genes needed for biological degradation of geosmin. In addition, sensory analytical methods (e.g. gas chromatography-mass spectrometry/olfactometry (GC-MS/O), proton transfer reaction-time-of-flight mass spectrometry (PTR-TOF-MS)) will be applied for the characterization and determination of factors that influence off-flavour formation in RAS. Organoleptic properties of the fish and its acceptance will be analysed through sensory evaluation and consumer testing. Furthermore, the impact of fish feed formulation on off-flavour formation will be explored by means of sensory analytics and experimental feed trials with off-flavour free and non-fishmeal-based ingredients.

Expected Results

The expected outcomes of this cluster of projects include identification of dominant off-flavours in water and fish from RAS; application of PTR-ToF-MS technology for off-flavour monitoring ; knowledge on which biotic and abiotic factors up- and downregulate the geosmin synthase gene; test of conventional and new prototypes of fish feeds for off-flavour modulation; identification of off-flavour degrading bacteria; and the analytical characterization of fish and it is relation to sensory perception and consumer acceptance.

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Discussion

Recirculating water in RAS may favour bacterial growth and the accumulation of the substances they produce, often including off-flavours with earthy and musty notes. Little is known about occurrence and biology of off-flavour producing bacteria, chemical characterization of the off-flavours and to which extent consumers accept tainted fish. RAS are extremely productive seafood farming systems that can operate throughout the year, in a variety of locations, such as next to major seafood markets, and without being affected by seasonality and environmental variables (Ahmed & Turchini, 2021). On the other hand, RAS are expensive and complex systems that require substantial and multidisciplinary knowledge. Moreover, the management of off-flavours must be eco-friendly, efficient and low-cost. The nutrition and culture environment are critical factors affecting volatile profiles of aquatic animals and are the variables that can be controlled by aquaculture producers (Jones et al., 2022). Hence, it is of great importance to holistically understand the system (biological, chemical and nutritionally) to develop robust methods that can efficiently prevent or manage the presence of off-flavours in RAS. In this project, innovative procedures to mitigate the prejudice of the tainting of RAS fish will be developed by identifying biological and chemical processes leading to production of the off-flavours. Solving off-flavour issues is key for the aquaculture industry, since consumers avoid buying fish products with an unacceptable taste. This approach could ensure high quality of fish products, which would lead to a higher consumer acceptance, in turn benefiting the entire RAS industry.

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DAILY RHYTHMS OF LIPID METABOLISM AND FATTY ACIDS PROFILE IN THE LIVER OF THE EUROPEAN SEABASS (*Dicentrarchus labrax*): INFLUENCE OF FEEDING TIME AND THE SEASON OF THE YEAR

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Introduction

Fish have daily rhythms synchronised mainly to the light/dark (LD) cycle. However, food may be a stronger synchroniser than light for some peripheral organs such as the liver. In aquaculture, nutrition is a major problem, mainly regarding lipid and protein sources and metabolism. Regarding the lipids, their metabolism seems to display daily rhythms in some species such as the zebrafish (*Danio rerio*) and the gilthead seabream (*Sparus aurata*). However, little is known about the existence of daily rhythms in lipid metabolism in other species and, moreover, the effects of the season and feeding time have not yet been studied in fish. The aim of the present research was to investigate the existence of daily rhythms in lipid metabolism (both mRNA expression and enzyme activity) and the lipid profile in the liver of the European sea bass (*Dicentrarchus labrax*). In addition, we tested the relative strength of two synchronisers, LD and feeding cycles, and whether these rhythms present seasonal differences.

Materials and methods

To this end, two samplings were carried out, the first one in December and the second one in June. For each sampling, fish were divided into two groups: one fed in the middle of the light phase (ML) and the other one fed in the middle of the dark phase (MD). Throughout the experiment, fish were kept under natural photoperiod and temperature conditions. After 30 days under the feeding regimes, samples were collected at different time points over a 24-h cycle (ZT0.5, ZT4, ZT7.5, ZT12, ZT16, ZT20 and ZT24.5 h), with ZT0 h belonging to light onset.

Result and discussion

The existence of daily rhythms in the expression of genes from lipogenesis and lipolysis processes was detected in both ML and MD groups and in both seasons. In general, the rhythms were more pervasive in December than in June. In addition, the expression levels were higher in December than in June in both groups. Different important fatty acids such as EPA and DHA displayed rhythms in both groups in December and June, with the amount of lipids being higher in June than December. The present study shows the existence of daily rhythms in lipid metabolism in the European sea bass and the influence of both feeding time and the season of the year on them. In addition, the results highlight the importance of considering circadian rhythms and the environmental factors influencing them when working with processes such as lipid metabolism and nutrition.

Acknowledgments

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FATTY ACIDS PROFILE OF LIVER, PLASMA, MUSCLE AND PERIVISCERAL FAT IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*): INFLUENCE OF FEEDING TIME AND SEASON OF THE YEAR

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Introduction

The animals synchronize their activity to light or darkness, showing daily rhythms in their activity pattern, so they are classified as diurnal or nocturnal. However, several fish species, like the European sea bass (*Dicentrarchus labrax*), can display either diurnal or nocturnal behaviour, which is called dualism. In the wild, European sea bass usually displays nocturnal feeding behaviour in winter and returns to diurnal feeding behaviour in spring and summer. In aquaculture, nutrition is a major problem, mainly regarding lipid and protein sources and metabolism. To date, the effects of the feeding time on lipid metabolism and composition have been little studied, especially considering also feeding patterns of dual behaviour as displayed by sea bass. The aim of the present research was to investigate the differences in the lipid profile in liver, plasma, muscle and perivisceral fat in European sea bass fed at two different feeding times and in two different seasons of the year, winter and summer.

Material and methods

Two samplings were carried out, the first one in December and the second one in June. For each sampling, animals were divided into two groups: one fed in the middle of the light phase (ML) and the other one fed in the middle of the dark phase (MD). Throughout the experiment, fish were kept under natural photoperiod and water temperature conditions. After 30 days under the feeding regime, samples of plasma, liver, muscle and perivisceral fat were collected and frozen immediately. The lipid profile was analysed by gas-liquid chromatography.

Result and discussion

The statistical analysis revealed significant differences in several fatty acids such as oleic, linoleic, α-linolenic, ARA, EPA and DHA between groups (ML and MD), season (December and June) and the interaction between both factors in muscle, liver, plasma and perivisceral fat. In muscle and liver, Total n6 and Total PUFA are higher in MD of December than in the other groups. In addition, in perivisceral fat the % of oleic, linoleic and α-linolenic is higher than in other tissues and the % of EPA, ARA and DHA are lower. In general, the % PUFA is higher in December MD and June ML. The change in activity must be taken into account in aquaculture and nutrition for a better accumulation of fatty acids and an increase in product quality.

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BEYOND BINDING – A DEEPER LOOK INTO MYCOTOXINS MITIGATION STRATEGIES FOR AQUATIC SPECIES

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Mycotoxin related pathologies in aquatic species have been constantly and globally described in scientific literature since the 1960's.

In recent years a shift towards increased use of plant origin materials and reduction of the animal components in aquatic feeds created a 'more-than-ever' risk of mycotoxicoses in aquaculture systems, which is now a globally growing and fast developing industry.

As their chemical structures vary considerably, mycotoxins cannot be classified as one group according to their mode of action, toxicology or metabolism (Gonçalves, et al., 2019). Moreover, the chemical and thermal stability of these molecules allows them to pass through the feed manufacturing procedures without reducing their toxic potential.
These characteristics together with the increasing wide range of cultured aquatic species create great challenges in development of modern mycotoxin mitigation strategies for aquaculture.

Aflatoxins (AF) were the first and most described mycotoxins in aquaculture. AF, to various extent, can be effectively adsorbed to suitable binders due to their planar molecular structure. The capacity, selectivity and specificity of the toxin binder are the main differentiating factors distinguishing these compounds from one another.

Other toxin groups cannot be bound in this manner as effectively and require different detoxification solutions such as biotransformation. Among these are trichothecenes (such as T-2 toxin and deoxynivalenol), zearalenone, and fumonisins (FUM), the last being one of the most commonly found mycotoxins.

FUM pose a major global concern, particularly in the Asian and Latin American regions where their prevalence in aquatic raw materials and compound feeds reaches levels of over 80% (Biomin, 2019). Li et al. (1994) described fumonisin B1 (FB1) as causing mortality in fish either by direct tissue damage, or by immunosuppression that results in higher sensitivity to infection. Chronic effects of FB1 include a decrease in body weight gain changes to haematological blood parameters decreased feed consumption, feed efficiency ratio, and impaired sphingolipid metabolism(Matejova, et al., 2017),.
Biotransformation by enzymes is one of the most modern forms of mycotoxin detoxification. Other biotransforming components include specific yeast and bacterial strains, some of these biological components have been proved to effectively detoxify specific mycotoxins.

In addition to toxins binding and biotransformation, specific plant and algal bioprotective compounds and extracts designed to support immunologic and metabolic processes demonstrated abilities to restore performance parameters when combined in the anti-toxin treatment.

The ubiquity of mycotoxins and their toxic potential to animal and humans calls for an evidence-based preventive solution. Important aspects such as antibiotic reduction, animal welfare and sustainable-economic aquaculture system all can be directly improved by choosing and applying the correct mycotoxin mitigation strategy.

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FITNESS FOR RELEASE - ARE THERE WAYS TO CIRCUMVENT MALADAPTATION CAUSED BY REARING TECHNIQUES DURING ONTOGENY?

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Introduction

Many fish populations are under pressure due to overfishing and environmental deterioration. They are frequently supported by release of cultured individuals. These releases are common practice in fisheries management, especially in waters that are subjected to angling pressure and for stock enhancement for instance in ocean ranching. Furthermore, in species conservation practices where the reproductive population is declining steadily, and effective population size has become critically low, *ex situ* practices are utilized to safeguard the remaining genetic diversity until the adverse impacts that led to the decline of the populations have been overcome, while hatchery release of progeny can be needed to allow self-sustaining populations to reestablish.

While the general suitability of the introduction of captive reared or transplanted fish has been increasingly questioned due to poor survival and adverse impact upon native populations, conditions exist where no alternatives for releases are available, and must be employed (for instance in reintroduction measures).

Results

The adverse impacts of confined rearing on post-release fitness and survival can strongly influence morphological, genetic, behavioral and physiological responses. Fish fitness as the ability to survive and reproduce in the harsh natural environment does not develop in the well-protected (disease and predator-free) culture environment. The monotonous nature of traditional hatchery practices, including high stocking densities, and predictability in feeding regimes, often results in a lack of environmental variability depriving behavioral responses linked to behavioral and neural deficits. As a result the fish reveal reduced survival in novel conditions. This has been exemplified in Gulf sturgeon (*A. oxyrinchus desotoi*) where survival following release reached only 20% of the fish that were originating from natural recruitment.

Over the past years, the adverse effects of such release practices have been the target of research and several means for the improvement of the rearing practices as well as the adaptation of release strategies have been considered wherever the releases have been determined as indispensable. For sturgeons, a first guideline to improve culture methods for release has been suggested.

Fish exhibit plasticity in neurogenesis depending upon the environment that they are reared in. While brain size is reported to be a result of the early life experiences with regard to the diversity of the environmental stimuli the neuronal interlinkage and brain structure adapts constantly. As such, transfer from monotonous environments into diverse ones even later in ontogeny has the potential to alter their cognition and adapt their physiological responses to challenges. Consequently, fish are sensitive to changes in environmental conditions and social interactions and thus are able to adopt skills to develop coping mechanisms provided by previous experience, i.e. do learn.

Discussion

Strategies to cope with behavioral depreviation - or even better - to prevent it at an early phase of development are strategies that have shown promising results in laboratory tests and following release. Such prevention or mitigation strategies can be applied as early as during reproduction, allowing mate choice and near natural incubation to optimize the resulting offspring. Among the mitigation strategies applied during embryonic development and rearing, streamside hatcheries are applied utilizing river water from the release site which are providing near natural conditions with regard to the environmental fluctuations while at the same time preventing excessive mortalities through predation. Alternatively, training schools prior to release have been shown to increase the adaptability of juveniles. Here, different stimuli or a combination of these can be used over a period of some 10 days to enhance the responsiveness of the fish to changing environments. It has been shown in Baltic sturgeon *A. oxyrinchus* that moderate environmental fluctuations such as temperature or light during the training phase alter the response of the fish with regard to the expression of heat shock proteins and increase the swimming performance. In current research it was shown that enhanced rearing conditions result not only in significant differences in behavior in laboratory experiments but also produce a significant difference in performance habitat choice and migration patterns) following release.

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As such the adaptation of rearing conditions to near natural variability or even the short-term exposition to environmental fluctuation improves the performance of the fish by enhancing their responsiveness and as such have the potential to increase effectiveness of releases and subsequent survival. Further studies are needed to develop methodologies to prepare hatchery-reared juvenile fish for fitness for survival in a highly competitive world, such as early recognition of potential predatory and effective escape reactions as well as early measures to support the development of the immune defense against pathogens known to occur in the natural habitat.

THE CONCEPT OF BALANCED FISH NUTRITION IN TEMPERATE EUROPEAN PONDS TO TACKLE EUTROPHICATION

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Introduction

Large fishponds (IR: 4.4–64 ha area, 1.3–3 m depth), predominant standing-waters in the land-locked central Europe, resemble temperate, shallow-lake ecosystems with immense contribution to regional eco-services (~74.5–100.6 million € country^{-1}), food security (≥50% aquatic protein/PUFA). Latest fitness check of EU-WFD revealed >70% of these waters failed to achieve good ecological status. Their *status quo* nutrient management are debated for eutrophication concerns, with focus on N:P-cycling (Roy et al. 2020a, 2020b). Till now, ecologists have mostly seen the problem of fishpond eutrophication through various perspectives. In line with the ecological nutrients stoichiometry theories predicting nutrient assimilation efficiencies, we show how in-vivo nutritional bioenergetics can strongly modulate nutrient translocation in aquatic systems. Especially how imbalanced stoichiometry of amino acids, digestible fractions of P, and carbohydrate energy (in fishponds) aggravate 'autochthonous' nutrient loading by carps.

In CEER fishponds, carps endure a counter-moving stoichiometry of food sources (natural food down; cereals up). As a result, strong shifts in nutrients reaching their gut per unit of ingested mass (e.g., digestible N or amino acids, bioavailable versus non-bioavailable P, digestible carbohydrate energy). Our core hypothesis was that such changing dietary scenarios across vegetative seasons have implications on carp's nutritional bioenergetics and raised the following questions: (a) is there a pattern in nutrient retention, faecal and metabolic excretion with the seasonally shifting diet components? (b) what are the deeper nutritional anomalies that aggravate or suppress nutrient losses from fish, making them source or sink nutrients? (c) is it possible to achieve a balanced diet and suppress fishes pumping out nutrients in reactive forms? – the central question.

Materials and Methods

A 11-month long experiment were conducted with lab-trained *Cyprinus carpio* (120-400 g) in a 12 tank (120 L tank^{-1}) Guelph system (19-21 °C, >4 mg L^{-1} DO, 6.8-7.3 pH) with provisions of 3-6 replicates per trial. Experiments were conducted in 2 phases: (a) digestibility trials (5 kg carp tank^{-1}) on cereals (corn, wheat, triticale; commonly used in fishponds) and lyophilised natural prey dry matter (daphnia, cyclops, chironomids; freshwater origin) @30% diet replacement method (basal diet = Skretting Carpe-F), 1% Y_2O_3 inert marker and passive faeces collection (every 4 h); (b) based on digestibility results (formula, NRC 2011), innovative diet formulations (compounded feed = wheat + natural prey @1Da:1Cy:1Ch 'by weight' + inert marker) were made on 'relative FCR basis' (calculated) simulating different scenarios of food availability for carps in regional fishponds (3 diet scenarios = 'BALANCED (cereals 2: natural prey 0.3)', 'LOW natural food, abundant cereals (2:0.1)', 'HIGH natural food, relatively low cereals (2:0.5)') – another round of digestibility trial (estimated digestible losses) + 5-week growth trial (2 kg carp tank^{-1}) was ran (estimated metabolic/ non-faecal losses, retention from initial and final whole body compositions of representative carps; thermal growth coefficient of stock). All feeds were cold-extruded (sinking) and fed 2% body weight basis.

Results

We show aggravated N, P loading by fish may occur both under high (≈beginning of the vegetative season) and low (≈end of season) zooplankton-zoobenthos availability. At beginning-season, the following phenomenon occurs in feeding fish: (a) high bioavailable P (P-homeostasis and digested-P urinated), (b) high PPR (renal stress≈ urinary PO_4^{3-} excretion), (c) renal EAA biosynthesis, e.g., arginine from abundant precursor NEAA proline (by-product≈ PO_4^{3-}), (d) low protein-sparing by low digestible carbohydrates (more protein catabolized≈ NH_4^+ excreted). Fish exhibit high but 'inefficient' N and P-retention under beginning-season diets. At end-season, the following phenomenon occurs: (a) insufficient, poor-quality protein (limited in lysine, isoleucine; rich in glutamic acid) results in poor N-deposition and aggravated NH_4^+ disposal, (b) highest P losses (poorest digestibility and discarding of already-digested P) in tandem with poorest N-deposition, (c) *de-novo* lipogenesis due to excessive starch, limitation of branched-chain amino acids for carbohydrate metabolism,

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obesity-inducing high omega-6: omega-3 fatty acids ratio in digested lipids. Protein accretion or growth almost ceases, fishes become fatty, and worst environmental loading of N, P happens in algae-reactive forms (NH_4^+, and PO_4^{3-}), under end-season diets. These novel observations were successfully validated against field metadata. We conclude highest ecosystem resource utilization efficiency and least N, P loading by fish are related to a balanced nutrition and managing fishes' satiety to graze (or spare) zooplankton-zoobenthos, enabling maintenance of clear-water phase and ecosystem services.

The present study provides a deep understanding how fish nutrition shapes excretion and eventually eutrophication in temperate shallow lake ecosystems like most large-sized European fishponds. We point out that improved ecosystem resource utilization efficiency (RUE) and tackling eutrophication may be achieved by 'bio-manipulating' temperate shallow lake ecosystems like large European fishponds towards a balanced fish nutrition (≈proposed approach). Besides extrinsic factors, the highest ecosystem RUE, highest ecosystem services and least internal N, P loading depends much on nutrition availability for fish in fishponds.

Discussions

Present supplementary feeding strategy in fishponds reflect a rather traditional, not very scientific practice of Central and Eastern Europe; not tuned towards improved resource (nutrient) use efficiency and nutrient bioeconomy. The managerial implication of this study is to improve present carp farming (feeding) practices for improved resource (nutrients) use efficiency, minimized N, P footprint and uncompromised production in CEER fishponds. The applied side of our proposed concept is in line with the state-of-the-art understanding of fish (animal) nutrition and in line with the plankton ecology group (PEG) principles to tackle eutrophication in shallow lake ecosystems, e.g., maintenance of clear water phase by zooplankton dominance. Large-bodied zooplankton (especially big daphniids) are the keystone group or species in shallow-lake ecosystems suppressing the eutrophication risks. Findings of 'nutritious pond' concept and present study hint that better retention efficiency and bioeconomy of food web derived N and P can be achieved simultaneously in fishponds through balanced "pond feeds" to complement the fluctuations in natural food base across distinct vegetative season windows. The idea is to also satiate the carps and spare natural food from being over-grazed; suppressing top-down and bottom-up effects.

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EFFECTS OF REPEATED ACUTE HYPOXIC STRESS ON PHYSIOLOGICAL RESPONSE IN RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

In a context of climate change changes in abiotic factors are taking place, among them. Oxygen that is a limiting factor both in the environment and in production systems, so reduction may become a stressor. In addition, climate change will increase the frequency of environmental hypoxic situations. The effects induced by hypoxic situations depend on the intensity of hypoxia, the duration of the stress event, or whether it is acute (short duration) or chronic (long duration). Under a stressful situation fish activate the hypothalamic-pituitary-interrenal axis (HPI) which triggers the release of cortisol that induces secondary and tertiary responses. The recovery of individuals subjected to stressors depends on their ability to modulate physiological, biochemical, and behavioural responses in order to maintain metabolic functions ans homeostasis.

The aim of this study is to determine the physiological response of rainbow trout under repeated hypoxic stress in different time points and different matrices, measured as cortisol levels. Sampling were 1, 6, 24 hours post stress, and samples were taken from plasma, skin mucus, water and scales.

Materials and methods

Rainbow trout juveniles were acclimated to AQUAB fish facilities (Universitat Autònoma de Barcelona, UAB), subjected to a 12L:12D photoperiod and 14ºC in a closed recirculation system. Fish were fed ad libitum with a commercial diet (Skretting). After the acclimation period, trouts reached a final body weight of 62,89 ± 11,10g and body length of 17,59 ± 1,09 cm. Fish were randomly divided in 5 different treatment groups, 2 control groups (absolute control and manipulated control) and 3 hypoxia groups: H1 that only received 1 hypoxic exposure, group H2 which received 2 hypoxic exposures and group H3 which received 3 hypoxic exposures.

Every exposure to hypoxia consisted in reducing the water oxygen level in the tanks from 8-9 mg/L to 2 mg/L by removing the aeration pumps and bubbling N_2 into the system. Then fish were left in the tanks for 1 hour and sample subsequently. Water oxygen levels were continuously monitored during the experiment. After the challenge, 9 fish per treatment, and time-point were euthanized with an overdose of Tricaine metanosuphonate (MS-222). Samples of blood, skin mucus, scales and water from the recovery tank were collected to analyse cortisol levels by ELISA. Plasma was isolated by centrifugation (10 min, 5000 rpm) and stored at -20ºC until analysis.

Results

Figure 1 shows the physiological response of cortisol levels at the different matrices analysed. Regarding plasma and skin mucus, the same pattern was observed, i.e., 1 hour post stress showed the highest levels in all groups. Regarding plasma, the difference between treatments was observed only with respect to the absolute control. On the other hand, with respect to skin mucus, greater differences can be observed between treatments, particularly between treatments. In the scales, when comparing the absolute control group with those that have undergone 3 hypoxic exposures, no significant differences could be established (Figure 1D). Finally, in terms of the cortisol values in the water, it is where more differences were observed between the different groups (Figure 1C). In addition, it should be added that the absolute control follows the same pattern between time-points as the plasma and skin mucus (Figure 1A and 1B), but the rest of the groups follow a different pattern.

Discussion

The data shows the typical physiological acute response to stress. It should be noted that the highest cortisol levels in all matrices were found 1 hour after stress. Furthermore, the results seem to indicate a circadian pattern of cortisol that can be observed in both plasma and mucus as previously observed in other experiments such *as Rance et al., 1982* and *Boujard et al., 1992,* among others. In contrast, this pattern could not be observed in water and scales. As far as the scales are concerned, no significant differences were observed, probably because deposition of cortisol in this matrix normally takes a longer time period. In terms of the physiological response to repeated exposure to hypoxia a progressive decrease in cortisol values was observed which may indicate an habituation of fish to this type of regular repeated stress.

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EFFECT OF CARBON SOURCE ON *Penaeus Vannamei* PRODUCTION UNDER BIOFLOC TECHNOLOGY (BFT)

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Biofloc technology is a system that reduces water consumption, and energy consumption and has the capacity to recycle the waste products into high-quality food that can be used by the species in production. In the current work, different carbon sources were tested at different proportions in a species of great commercial value such as the white shrimp (Penaeus *vannamei*), observing that molasses with C/N ratios of 14:1 and 18:1, provided the best survival results and growth, likely, thanks to a differential microbial community.

Introduction

The production of white shrimp (*Penaeus vannamei*) is the most important sector of aquaculture with a production in 2020 of 4,966 million kg and a commercial value of 28,782 million U$. Traditionally, shrimp has been produced in semi-intensive systems, however, intensive and super-intensive systems in RAS and biofloc (TBF) technology are increasing, with much higher densities (250-500 shrimp/m^2). TBF is based on the principle of recycling waste nutrients, particularly nitrogen, into microbial biomass that can be used *in situ* by the animals produced or collected and processed into feed ingredients.

Material and Methods

A classic biofloc system requires external contributions from an exogenous carbon source, both its composition and its proportion in relation to nitrogen (C/N ratio) being important. In the current work, 3 experimental groups were designed according to the carbon source used: molasses, brewer's yeast plus a source mainly of di- and polysaccharides (77%), and a polymer (1,4-butylene succinate; PBS) plus a source of carbon formed mainly of monosaccharides (68%). Within each source, 5 different C/N ratios have been tested: 26/1, 22/1, 18/1, 14/1, and 10:1. Each ratio had 4 replicates, so a total of 20 tanks per carbon source.

Once the shrimps reached the average weight of 0.5 g, they were introduced into the tanks at final densities of 350 shrimp/m^2 (super-intensive production conditions). The animals were housed in 90 L tanks at a salinity of 21±0.14 g/L, temperature of 28 ºC, pH 7.5-8.5, oxygen >5 mg/L, and alkalinity >150 mg/L. Ammonium, nitrite, nitrate, alkalinity, or phosphate values were measured weekly using colorimetric kits in a spectrophotometer (Hanna instruments). Settleable solids were analysed using the Imhoff cone and total suspended solids (TSS) after filtering 80 ml of water through a microfiber filter. In order to have a better understanding of the C/N ratio, the total carbon and nitrogen of the biofloc particulate matter were analyzed weekly by direct combustion, using a Leco CN628 Elemental Analyzer. In addition, to evaluate the evolution of the biofloc bacterial community in the different experimental groups, microbial counts (culturable and cytometric) were performed at different points of the experiment: initial and intermediate (1.5 g mean weight). To carry out the microbial diversity analysis, Shannon index, functional richness, and metabolic characterization (AWCD) were performed. All experimental groups were fed with a standard formulation feed for an intensive production system, which includes 35% crude protein (CP) and 10% fat. Every two weeks shrimp were sampled to assess both, survival and weight gain (Kuhn et al., 2010).

Results and discussion

The values of temperature, oxygen, pH, salinity, and alkalinity were found within the pre-established values. Higher C/N ratios were registered when the carbon source was molasses, followed by yeast and polymer. About the evolution of nitrogenous compounds, as expected, the ammonium, nitrite, and nitrate peaks followed each other, being generally higher in the molasses group (Figure 1A-1B). Nevertheless, the highest final weight was reported in molasses, without significant differences concerning survival (Figure 1D-1E). Consequently, despite the higher nitrogen toxic level in the water, molasses biofloc was able to provide a better growth performance. Regarding the ratios, the intermediate values from 14/1 to 22/1 provided the best results, both for production values and water quality.

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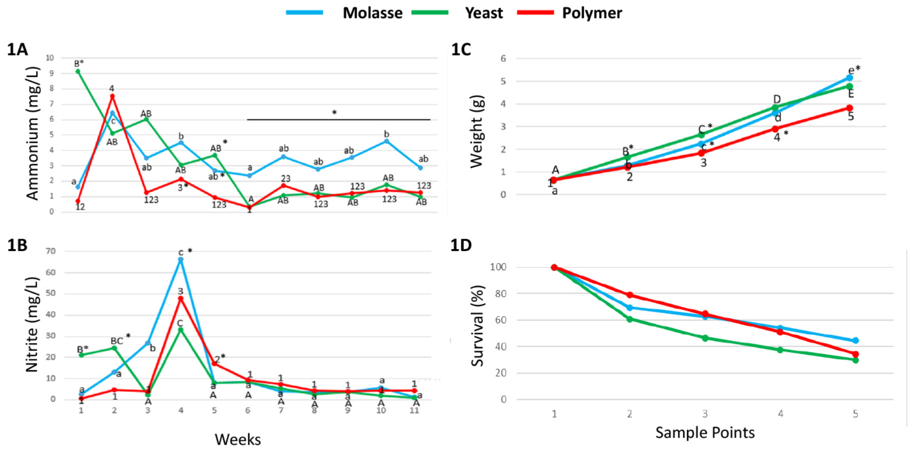

Figure 1. Evolution (mg/L) of ammonium (NH_4+), 1A; nitrite (NO_2-), 1B; average weight (1C) and survival (1D) by carbon source. Lower case letters represent significant differences through the experimental period for the molasses group, capital letters for yeast group, and numeration for polymer group. Asterisk (*) represents differences among carbon sources at the same time point.

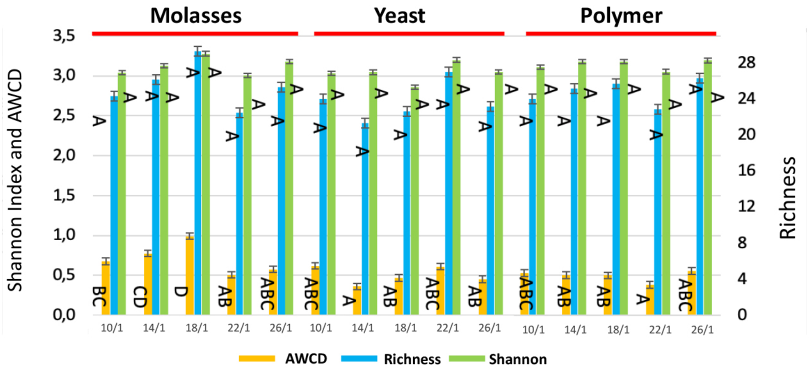

Figure 2. Shannon index, functional richness, and metabolic characterization (AWCD) of different experimental groups. Letters represent significant differences at 45 days of experimentation.

The results of the studies of the bacterial populations indicate a predominant marine heterotrophic microbiota in all the experimental groups, which was expected because the tanks were kept in the dark. Regarding the total and nitrifying bacteria counting, the molasses group registered the highest values of total bacteria at 45 days, although without differences regarding nitrifying bacteria. Concerning microbial diversity, no differences at 45 days of experimentation were found respect richness and Shannon index, but the microbial community of molasses group showed a more diverse metabolism, especially the intermediate groups (Figure 2). These results could explain the best growth performance for the intermediate ratios of molasses group, despite the highest levels of ammonium and nitrite.

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TAMING HOLOTHURIANS FOR THEIR USE IN INTEGRATE MULTITROPHIC AQUACULTURE: FAILURES, SUCCESSES AND PERSPECTIVES

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An efficient integrated multitrophic aquaculture (IMTA) requires species capable of effectively converting particulate waste into biomass (Chopin et al., 2012). These species, called detritivores help mitigate the benthic impacts of aquaculture farms, once extracted of the system, and represent, ideally, an additional income to the production. Sea cucumbers are interesting candidates for IMTA due to their prevalence in the marine environment, their diversified diet and their economic value (Zamora et al., 2018). Yet scarce information is available regarding the capacities of European species to be stocked and reared underneath aquaculture cages. There is therefore a growing scientific effort in Europe, including in France, to get more insight on the biology, the reproductive physiology, the embryonic development, the feeding behavior, and on their growing capacities in captive environments (David et al., 2020; Laguerre et al., 2020; Sadoul et al., 2022)to date little is known about the nutritional requirements of East Atlantic and Mediterranean species. In this study, we propose a "natural population"-oriented approach to characterize food sources, digestive efficiency and resources allocation based on the composition of pigments and fatty acids (FA. Two French projects (EPURVAL2 and HOLOFARM) have thus investigated possibilities of using local deposit-feeding sea cucumber species, *Holothuria forskali* and *Holothuria tubulosa*, for bioremediation of aquaculture particulate waste. This presentation aims at sharing to the aquaculture community the failures, successes and perspectives highlighted by both projects. Among others, difficulties with escapees, high mortalities, reduced growth in captive conditions, and biometrics will be shared along with possible solutions. Both projects have allowed significant breakthrough in the use of these species for bioremediation and estimation of their growing capacities using bioenergetic modeling. Also, we have made substantial advances in our capacities to reproduce and raise juveniles, which seem to be, as a conclusion of both projects, the key to efficiently bioremediate aquaculture waste. Research perspectives regarding stocking densities, repeatable reproduction, and identification of geographically effective sites for their use will also be discussed.

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EVALUATION OF INNOVATIVE REARING TECHNIQUES FOR OPTIMIZING EFFICIENCY AND QUALITY OF OYSTER PRODUCTION

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Oysters have been cultured for millennia all around the world, and represent now the leading molluscan species produced in volume (Botta et al., 2020). While France is still the leading European country for oyster production, annual production has generally decreased over the last two decades ("Graph'Agri," 2021). The use of innovative technologies adapted for our rearing conditions can help improve rearing efficiencies, quality of the product and therefore favor sustainability of the production. Here, we investigated the potential benefits of 1) a co-culture of Pacific oyster, *Crassostrea gigas*, with the grazing gastropod *Littorina littorea* vs a monoculture of *C. gigas*, 2) offshore compared to classic intertidal culture and/or 3) oyster basket compared to oyster mesh bag. We tested all conditions in a full cross factorial study over three months of rearing. Effects on growth, aspect, flesh percentage, and fatty acids profiles were investigated. We also compared the total fouling between conditions. Results show that off-shore rearing is greatly beneficial for getting the best quality of the final product, in terms of aspect, survival and flesh content. The association with *L. littorea* showed great potential for reducing fouling and ultimately potentially favoring growth. Fatty acids analyses highlighted the amazing contents in omega-3 (n-3) long-chain polyunsaturated fatty acids, particularly important for human nutrition, in all products but more importantly in oysters reared off-shore. Altogether these results, show the great potential of these techniques for increasing the efficiency and quality of oyster products while reducing work load of professionals.

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EFFECTS OF ALGAE-BASED DIETS ON SHELF-LIFE EXTENSION OF TURBOT (*Scophthalmus maximus*) FROZEN FILLETS

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Introduction

Turbot (*Scophthalmus maximus*) is an emerging fish candidate for diversifying the aquaculture in the Southern Europe. This flatfish can be commercialised both fresh and frozen owing to its leanness, since high lipid content in other fish species is considered a major constraint for freezing. Moreover, the presence of polyunsaturated fatty acids in fish fillets contributes for increasing susceptibility of lipids to oxidative processes, decreasing both shelf-life and sensorial quality. Therefore, the prevention of lipid oxidation constitutes an effective approach for delaying fish spoilage, being one of the major current challenges for the seafood industry. Algae are rich in a wide variety of bioactive compounds, such as carotenoids and polyphenols among others, which provide remarkable antioxidant and antimicrobial activities (Nakagawa *et al.,* 2012). Thus, several studies have broached the application of algae as natural preservatives for fish products. However, to date the potential effects of algae on objective quality parameters in turbot remains scarcely explored. In this context, this study explores whether the administration of *Ulva rigida* meal or a blend of microalgae at low dietary inclusion level could influence the objective quality parameters of *S. maximus* frozen fillets as well as extend their shelf-life during cold storage.

Materials and methods

Experimental diets were elaborated at the Ceimar-Universidad de Almería facilities (Service of Experimental Diets, https://bit.ly/3wdO03h, Almería, Spain). Three iso-nitrogenous (50.5%, DW) and iso-lipidic (16.5%, DW) experimental feeds were formulated: **U5** containing 5 % of *U. rigida* meal, **M5** containing 5 % of a blend of freshwater and marine microalgae, and a free-algae diet used as control (**CT**). Additionally, a commercial aquafeed specific for this species (**COM**) was tested. Turbot juveniles were randomly distributed in 12 tanks, and fed at a rate of 2% of the total biomass during a 180-d period. At the end of feeding trial, 30 specimens per dietary treatment were withdrawn. After slaughtering, specimens were gutted, filleted and packed in transparent sterile polyethylene bags. These bags were directly stored at -20 ºC for an extra period of 150-d with the aim of evaluating changes in objective quality parameters throughout the frozen fillet shelf-life. Samples were extracted from each lot at 1, 3, 6, 8 and 10 days post-thawing (dpt), and hardness, pH, water holding capacity (WHC), skin pigmentation and lipid oxidation were determined at each sampling time. In addition, at the initial sampling point muscle proximal composition was determined.

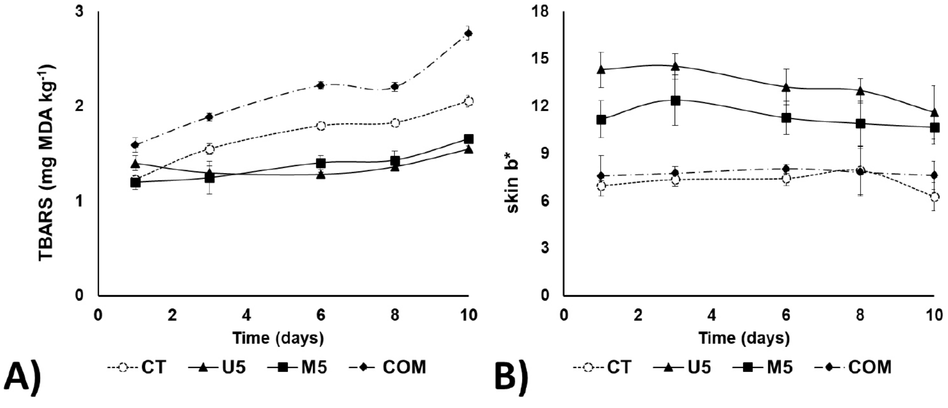

Figure 1. Time-course of A) TBARS muscle content and B) skin b* parameter of turbot frozen fillets. Dietary codes: CT: control; U5: 5% *U. rigida*; M5: 5% microalgal blend; COM: commercial turbot aquafeed. Values are expressed as mean ± sd (n=6).

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Results and discussion

The chemical composition of fillets was influenced by dietary treatments (P≤0.01), showing an increase of muscle lipid content in specimens fed on COM diet. U5 and M5 fillets tended to show slightly lower lipid values compared to CT, but without significant difference (P=0.17). Overall, algae inclusion in diets (U5 and M5 batches) decreased muscle lipid oxidation during cold storage time, extending frozen fillet shelf-life (P≤0.01, Figure 1.A). This protective effect is extremely useful considering that freezing and thawing processes accelerate fish deterioration, shortening fillet shelf-life. Regarding skin pigmentation, results obtained evidenced that a* and b* parameters were modified by dietary treatments (P≤0.01 and ≤0.01, respectively). In general, the skin of algae-supplemented fed fish, compared to CT and COM batches, was greenish (a*) and yellowish (b*), mostly due to differences in b* parameter observed after *U. rigida* dietary inclusion (Figure 1B).

Aside from skin pigmentation and oxidative status, textural parameters represent a crucial feature of fish muscle, both in terms of consumer's acceptance and shelf-life. In this context, algae inclusion in aquafeeds did not affect neither hardness nor WHC parameters of muscle. However, cold storage time caused a decrease of the percentage of water retained in the muscle in COM and M5 batches (P≤0.01). Regarding pH parameter, values increased significantly during 10 dpt in all experimental batches, owing to the emergence of alkaline compounds from amino acid bacterial degradation (Masniyom *et al.,* 2002). Despite this fact, significant lower pH values were observed in U5 and M5 fillets compared to CT and COM (P≤0.01) during post-thawing period.

Conclusions

The results confirmed either microalgae or *Ulva rigida* administered at low inclusion level (5%) in grow-out aquafeeds has positive impact on different aspects related to antioxidant activity and skin pigmentation in turbot. In addition, these functional effects could be key on consumer acceptance and the shelf-life extension of frozen fillet.

Acknowledgements

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INFLUENCE OF MICROALGAE-BASED ADDITIVES IN FINISHING DIETS ON THE SHELF-LIFE OF SEABREAM FROZEN FILLETS

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Introduction

Fillet quality attributes and commercial shelf-life heavily rely on fish species-specific factors, but also on the utilization of commercial additives aimed at delaying deterioration of fish. Both microbiological and biochemical phenomena jeopardize the quality of the final product, and therefore, they represent the target of most of the food additives. However, the use of chemically synthesized substances on the final product is limited both by legal regulations and by the low acceptability by consumers of this strategy. The consequence is that a limited array of such preservatives is available for the seafood industry, while processed fish formats are increasing in the market, not least those involving extended storage periods. In this regard, frozen fish involve long-term storage, in which biochemical deterioration is still possible. Namely, lipid peroxidation and protein degradation are among the most known phenomena that can alter fillet quality. Scarce research has been conducted aimed at controlling oxidative deterioration in fish during frozen storage based on dietary strategies, and therefore, there is considerable room for innovation in this field. In this regard, a promising strategy is represented by the utilization of functional aquafeeds enriched with bioactive additives included at low percentage (less than 10%). An emerging application for these innovative feeds is their administration at the end of the production cycle (finishing feeds), since variations in diets are rapidly reflected in fillet quality (Grigorakis *et al.,* 2002). Among the potential additives with this aim, plant extracts, prebiotics, and microalgae stand out, all of them with potentially valuable physiological effects on fillet pigmentation or oxidation of fillet lipids. However, a possible disadvantage associated with the use of prebiotics and microalgae as functional additives consists in the existence in some cases of a thick cell wall, which can limit the bioavailability of the potential bioactive inner compounds. The use of biotechnological treatments, such as enzymatic hydrolysis with fibrolytics enzymes, could overcome this limitation.

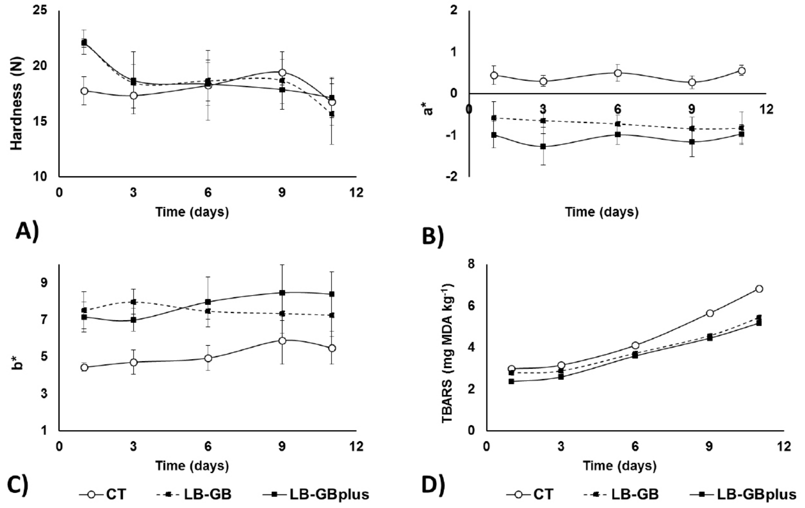

Figure 1. Time course of A) hardness, B and C) a* and b* skin colour parameters, and, D) lipid oxidation in frozen seabream fillets. Values are mean ± sd (n=4).

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Materials and methods

Three iso-nitrogenous (42.5%, DW) and iso-lipidic (17.5%, DW) experimental feeds were formulated at the CEIA3-Universidad de Almería facilities (Servicio de Piensos Experimentales, http://www.ual.es/stecnicos_spe; grants EQC2018-004984-P and EQC2019-006380-P). LB-Gb diet contained a 10% of LB-Goldbream, a commercial microalgae-based additive provided by Lifebioencapsulation S.L. This product consists of a blend of microalgae and prebiotics. A second diet, designed as LB-Gbplus diet, was also prepared; similar to LB-Gb diet, except for the fact that the blend of microalgae and prebiotics was hydrolysed enzymatically. In addition, a third diet, additive-free, was used as the control batch (CT). A feeding trial was carried out at the aquaculture facilities of "Servicios Centrales de Investigación en Cultivos Marinos" (SCI-CM, CASEM, Universidad de Cadiz, Spain). Gilthead seabream specimens (180.0 ± 10 g b.w.) were randomly distributed in 9 tanks, and the experimental diets were offered at 2% of the biomass during a 41-d period. At the end of feeding assay, 15 fish per treatment were withdrawn. Immediately after slaughtering, specimens were gutted, filleted, packed in transparent sterile polyethylene bags, and stored at -20 ºC for six moths. Afterwards, fillets were thawed, and then transferred into a cold chamber (4 ºC) for an extra 11-d period. Samples were withdrawn from each lot at 1, 3, 6, 9 and 11 days post-thawing, and texture profile analysis (TPA), pH, water holding capacity (WHC), skin pigmentation and lipid oxidation were determined at each sampling time.

Results and discussion

During post-thawing cold storage, hardness decreased markedly in all experimental lots ($P<0.01$); however, at the beginning of the trial additive-enriched diets were responsible for fillets with significantly higher hardness than controls ($P<0.01$, Figure 1A). Roughly, gumminess, chewiness and WHC followed a tendency similar to that observed for hardness, whereas springiness, cohesiveness and resilience, and well as muscle pH. On the other hand, skin colour (pigmented fillet side) showed significant differences for a* (redness) and b* (yellowness) parameters attributable to the inclusion of the commercial additives in diets ($P<0.01$ and $P<0.01$, respectively). Roughly, LB-Gb and LB-Gbplus fillets displayed lower a* and higher b* values than CT fillets up to 6 days post-thawing (Figure 1B and C). In addition, significant differences between both additive-enriched diets were found in a* parameter (lower values for LB-Gbplus than for LB-Gb batch). Regarding storage time, a* and b* values remained stable from the beginning to the end of the assay ($P=0.36$). Lipid oxidation depended clearly on storage time in all experimental groups, as evidenced by the significant increase of TBARS throughout the storage period ($P<0.01$, Figure 1D). However, although the most contributing factor to TBARS concentration was storage time, additive-enriched diets decreased significantly ($P<0.01$) fillet lipid oxidation at any sampling time, compared to CT batch.

Conclusions

Overall, the results obtained in the present study showed that the incorporation of LB-Gb and LB-Gbplus in finishing aquafeeds yielded favourable effects on *S. aurata* frozen fillet quality, namely on textural, pigmentation and oxidation parameters.

Acknowledgements

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EFFECTS OF DIETARY SUPPLEMENTATION WITH ACID OILS FROM SOYBEAN AND OLIVE POMACE ON HEPATIC AND INTESTINAL HISTOLOGY IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

Vegetable acid oils (AO), by-products from the edible oil refining industry are characterized by having a similar fatty acid (FA) composition to that of their corresponding crude oil, free fatty acid (FFA) being the major component (> 40 % of lipid classes) (Varona et al., 2021). Its high energetic value makes them promising candidates as energy sources into aquaculture feed, enabling an upcycling of these by-products. The effect of inclusion of AO in fish feed on growth and digestibility has scarcely been studied (Trullàs et al., 2016 a,b; Verge-Mèrida et al., 2022). The aim of the present study was to investigate the histological effects of the inclusion of soybean and olive pomace AO on hepatic and intestinal tissue in European seabass (*Dicentrarchus labrax*).

Materials and Methods

A total of 300 individuals (101.5 ± 0.4 g, initial weight) were distributed into 15 tanks. Five were fed for 100 days, differing in the added fat source (25% fish oil, 75% experimental oil): S (crude soybean oil), SA (soybean-sunflower acid oil), O (crude olive pomace oil) or OA (olive pomace acid oil) and a control diet containing with only fish oil (FO diet). At the end of experimental period, samples of the liver and anterior part of intestine were collected from 4 fish/tank (3 tanks/experimental diet; 12 fish /treatment) and processed using a routine histological procedure in paraffin. Serial sections were stained with hematoxylin-eosin. In liver, also the size and the degree of vacuolization of hepatocytes were analyzed according to Kowalska et al. (2011) and Figueiredo-Silva et al. (2005) using a five-degree semi-quantitative scale. In anterior intestine, a morphometric evaluation was carried out, giving attention to the enterocyte height (300 measurements/treatment; 10 folds/fish), the goblet cell number (10 folds/fish; 120 folds/treatment) and the widening degree of lamina propria (LP) in the intestinal folds using a semi-quantitative system adapted from Úran (2008) and Millán-Sorribes et al. (2020). Data were statistically treated using a one-way ANOVA and analyzed for statistical significance using Tukey's multiple range test ($P<0.005$).

Results and Discussion

No significant differences in hepatic morphohistology and hepatocyte size were observed among the different treatment groups ($P>0.05$) (Table 1). However, lipid accumulation observed tended to be higher in the soybean oil diets (S and SA) similar to that described for this specie and rainbow trout (Figuereido-Silva et al, 2005). None of these changes were considered pathological due to the lack of extensive hepatic necrosis (Saraiva et al, 2015)

Table 1. *Hepatic and intestinal parameters of European seabass fed experimental diets.*

Parameter	FO	S	SA	O	OA	SEM	P-value
Liver							
Hepatocyte area (μm^2)	16.63	19.14	21.73	18.92	18.19	±1.62	0.274
Vacuolization degree	2.11	2.81	3.23	2.48	2.16	±1.46	0.226
Anterior intestine							
Enterocyte height (µm)	4.56^{b}	4.92^{ab}	3.78^{c}	5.20^{a}	5.22^{a}	±0.145	<0.001
Goblet cell number(/100µm)	4.76^{ab}	4.30^{b}	5.68^{a}	3.96^{b}	3.79^{b}	±0.299	<0.001
Widening degree of LP	2.02^{ab}	2.18^{a}	2.12^{a}	2.05^{ab}	1.79^{b}	±0.085	0.016

Absence of superscript letters indicates no significant difference between treatments ($P>0.05$). FO: Fish oil diet; S: Soybean oil diet; SA: Soybean acid oil diet; O: Olive pomace oil diet; OA: Olive pomace acid oil diet. LP: Lamina propia. SEM: Standard error of the mean (n=12).

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In anterior intestine, no pathological changes in the epithelium were observed among treatment. When crude oil diets (S and O) are compared with FO diet only statistically differences were obtained for enterocyte height, being higher for O diet (Table 1). Although a higher enterocyte height had been related to a higher absorption of nutrients, no differences in lipid apparent digestibility was obtained of using the same diets (Verge-Mèrida et al, 2022). When S was replaced by SA, lower enterocyte height and a higher globet cell number was observed, which would point to a lower absorption and a general gut inflammation (Torrecillas et al, 2019), respectively. In contrast, no significant differences were obtained for these histological parameters when OA replace O. These results agree with the differences in saturated fatty acid digestibility observed when SA replace S, but not in olive pomace oils (O and OA), although a lower total lipid digestibility was obtained for AO compared to its corresponding crude oils (Verge-Mèrida et al., 2022). No effect of crude and experimental oils has been obtained in the widening of the LP which can indicate the absence of a non-infectious sub-acute enteritis due to an infiltration of inflammatory cells (Urán, 2008).

From the results, it can be concluded that the effect of the inclusion of acid oils in the diet of European seabass on liver and intestinal histology differs between the two fat sources used (soybean oil and olive pomace oil), although their inclusion does not appear to compromise intestinal and liver integrity.

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ASSESSMENT OF APPARENT DIGESTIBILITY OF DIETS AND INGREDIENTS FOR GILTHEAD SEABREAM *Sparus aurata*, SOBAITY BREAM *Sparidentex hasta* AND ASIAN SEABASS *Lates calcarfier* UNDER THE RED SEA CONDITIONS

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Introduction

Fish feed is the principal operating cost in the production of fish and the main protein and energy source has traditionally been fish meal and fish oil. Research has concentrated on replacing fish meal and fish oil with cheaper ingredients of either animal or vegetable origin. Knowledge of the apparent digestibility coefficients (ADCs), of these various ingredients is a basic requirement for formulating diets. ADCs, provide estimates of nutrient availability in foods, and can be used to select ingredients that optimize the nutritional value and cost of formulated diets (Lupatsch *et al.*, 1997). Therefore, this study designed to compare the digestibility of a series of diets and ingredients when fed to Gilthead seabream, Sobaity bream and Barramundi under the Red Sea environmental conditions.

Materials and Methods

The diet-substitution digestibility method was used in this study (Glencross et al. 2007). ADCs were determined for dry matter (DM), crude protein (CP), crude lipid (CL), gross energy (GE), and amino acids of the available local ingredients in KSA. A basal diet (diet 1; 500 g kg^{-1} DM protein, 200 g kg^{-1} DM lipid) and test diets (of 14 different diets consisting of 70% basal diet and 30% of the feedstuff) were used with 1% Yttrium oxide as an external marker. The composition details of each diet is presented in (Table 1). The experimental fishes, averaging 150 to 300 g, was stocked in 850 L tanks at a density of up to 30 fishes per tank, under Red Sea conditions; seawater with salinity of 42 ppt; dissolved oxygen 7.33 ± 0.18 mg L^{-1} and water temperature at around 28ºC to 30ºC. Feces were collected in triplicate by stripping based on best timing for digestion.

Results

There were several significant differences among the digestibility parameters of the experiment diets (Table 1). Diet dry matter digestibility was poorest in the FM-TUNA diet (0.439) when fed to Gilthead seabream, and best recorded results for the same diet when fed to barramundi (0.606). Dry matter digestibility was highest in the WHEAT-GLUTEN diet when fed to barramundi (0.743), though this was significantly higher when fed to Gilthead seabream (0.684) and Sobaity bream (0.642). Diet protein digestibility was poorest in the FM-TUNA diet with (0.809) when fed to Gilthead seabream, while it was highest in the WHEAT GLUTEN diet when fed to Barramundi as shown (0.934), though this was not significantly differed when same diet fed to Gilthead seabream and Sobaity bream as (0.930) or (0.919), respectively. Diet energy digestibility was poorest in the WHEAT MEAL diet (0.668) when fed to Sobaity bream, which was significantly poorer when fed to Barramundi (0.727) and Gilthead seabream (0.754).

Table 1. Composition and Digestibility coefficients (means) of the experimental ingredients (all values are g kg^{-1} DM)

Ingredient type	BASAL	FM-UMP	FM-ARASCO	FM-LT70	FM-TUNA	CGM-ARASCO	SOY MEAL	SOY ARASC	WHEAT MEAL	WHEAT GLU	SPC	SARD Oil	BP FishOil	Rape seed Oil	Poultry Oil
Treatment diets	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12	Diet 13	Diet 14	Diet 15
Moisture	7	6.4	6.1	6	6.2	6.2	7.2	8	8	6.9	7	7	7.1	8.2	7.5
Dry matter (g/100g)	93	93.6	93.9	94	93.8	93.8	92.8	92	92	93.1	93	93	92.9	91.8	92.5
Crude protein	45.6	50.4	50	50.5	48.7	50.4	45.5	44.7	34.5	53.3	48.4	41.8	42.6	39.7	42.7
Crude fat	11.9	11	9.5	9.86	12.4	12.3	9.93	9.77	9.29	10.5	8.73	21.1	18.4	18.9	23.1
Ash	6.1	10.3	10.2	9.5	10.9	3.8	5.1	4	2.1	1.1	4	2.4	5	5.4	5.4
Carbohydrates	36.4	28.3	38.5	30.14	28	33.5	39.47	41.53	54.11	35.1	38.87	34.7	34	36	28.8
Gross Energy (kJ/g DM)	21.75	21.13	18.97	21.027	21.235	22.54	21.48	21.59	21.17	22.79	21.59	24.20	23.20	23.06	24.18
Diet digestibilities															
Gilthead seabream															
Dry matter	$^{a}0.574^{b}$	$^{a}0.560^{b}$	$^{b}0.477^{c}$	$^{a}0.536^{b}$	$^{b}0.439^{cd}$	$^{a}0.527^{b}$	$^{a}0.511^{b}$	$^{a}0.527^{b}$	$^{a}0.541^{b}$	$^{b}0.684^{a}$	$^{a}0.509^{b}$	0.628	0.606	0.623	0.625
Protein	$^{a}0.873^{b}$	$^{a}0.863^{b}$	a0.822^{de}	$^{a}0.850^{bc}$	$^{a}0.809^{de}$	$^{a}0.843^{cd}$	$^{a}0.880^{b}$	$^{a}0.875^{b}$	$^{a}0.883^{b}$	$^{a}0.930^{a}$	$^{a}0.877^{b}$	0.896	0.892	0.897	0.901
Energy	$^{b}0.780^{b}$	$^{a}0.802^{b}$	b0.722^{c}	$^{b}0.778^{b}$	$^{b}0.736^{c}$	$^{b}0.758^{bc}$	$^{b}0.750^{bc}$	$^{b}0.751^{bc}$	$^{b}0.754^{bc}$	$^{a}0.853^{a}$	$^{a}0.740^{c}$	0.827	0.815	0.819	0.823
Sobaity bream															
Dry matter	$^{a}0.511^{b}$	$^{a}0.502^{b}$	$^{a}0.454^{c}$	$^{a}0.541^{b}$	$^{a}0.492^{b}$	$^{a}0.513^{b}$	$^{a}0.496^{b}$	$^{a}0.510^{b}$	$^{a}0.442^{c}$	$^{b}0.642^{a}$	a0.547^{b}	0.618	0.593	0.575	0.585
Protein	$^{a}0.868^{b}$	a0.833d	$^{a}0.823^{e}$	$^{a}0.854^{c}$	$^{a}0.822^{e}$	$^{a}0.849^{c}$	$^{a}0.878^{b}$	$^{a}0.881^{b}$	$^{a}0.870^{b}$	$^{a}0.919^{a}$	a0.871^{b}	0.897	0.891	0.883	0.890
Energy	$^{b}0.742^{b}$	$^{a}0.754^{b}$	$^{a}0.695^{d}$	$^{a}0.764^{b}$	$^{a}0.740^{b}$	$^{a}0.738^{b}$	$^{a}0.713^{c}$	$^{a}0.718^{c}$	$^{b}0.668^{e}$	$^{a}0.812^{a}$	$^{a}0.736^{b}$	0.808	0.781	0.784	0.797
Barramundi															
Dry matter	$^{a}0.682^{ab}$	$^{a}0.697^{ab}$	a0.642^{ab}	$^{a}0.717^{a}$	$^{a}0.606^{cd}$	$^{a}0.634^{ab}$	$^{a}0.656^{ab}$	$^{a}0.649^{ab}$	$^{a}0.526^{de}$	$^{a}0.743^{a}$	$^{a}0.614^{cd}$	0.688	0.680	0.666	0.680
Protein	$^{a}0.904^{a}$	$^{a}0.886^{b}$	a0.860^{bc}	$^{a}0.901^{b}$	$^{a}0.837^{cd}$	$^{a}0.875^{b}$	$^{a}0.904^{a}$	$^{a}0.905^{a}$	$^{a}0.884^{b}$	$^{a}0.934^{a}$	$^{a}0.884^{b}$	0.901	0.900	0.891	0.901
Energy	$^{a}0.897^{a}$	$^{a}0.858^{a}$	a0.768^{c}	$^{a}0.839^{a}$	$^{a}0.765^{d}$	$^{a}0.837^{a}$	$^{a}0.785^{b}$	$^{a}0.800^{b}$	$^{a}0.727^{d}$	$^{a}0.847^{a}$	$^{a}0.753^{d}$	0.818	0.800	0.834	0.813

Different presuperscripts within columns indicate significant differences between means among the same digestibility parameters between fish species, but not between different digestibility parameters or between different ingredients ($P < 0.05$). Different postsuperscripts within rows indicate significant differences between means among ingredients, but not between digestibility parameters or diet type ($P < 0.05$).

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Ingredient protein digestibility was poorest in the WHEAT MEAL (0.401) when fed to barramundi and highest in the WHEAT GULTEN diet when fed to Gilthead seabream as (0.985), though this was not significantly differed when fed to Barramundi (0.952) and Sobaity bream (0.966). The overall range in ingredient protein digestibility values was higher for Barramundi (0.841) than Gilthead seabream (0.834) and Sobaity bream (0.832). Also, the overall range in ingredient energy digestibility values was higher for Gilthead seabream (0.736) than Sobaity bream (0.727) and Barramundi (0.576).

Discussion

Evaluation of apparent digestibility coefficient for dietary diets and ingredients in fish nutrition is contribute better to assess their potential nutritional value and nutrients availability through short term digestibility trials which help in optimum diet formulation. In this regard, chemical composition and quality of meals differ considerably depending on raw materials and processing methods. Energy content of the feed is considered to be the main factor controlling feed consumption in finfish (Boujarda et al., 2004). In this study, there was a strong correlation between Gilthead seabream and Sobaity beam and Barramundi for diet DM digestibilities as well as protein digestibilities. This provided responses of different species when subjected to same type of diets and feed.

Conclusions

These findings may put on the table a new emerging evidence considering the Red Sea environmental condition specifically higher salinity and temperature as an issue considering different digestibility responses based on the behavioral nature of all the species tested being carnivores. Diets then could be formulated on the basis of digestibility of individual ingredients and protein could be recommended to use as alternative protein source in all diets tested for the species.

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EFFECT OF DIETARY NANO-CURCUMIN ON GROWTH, IMMUNITY, HEAT STRESS RESISTANCE AND STRESS INDICATORS OF NILE TILAPIA (*Oreochromis niloticus*)

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Heat stress is one of the consequences of climate change which has devastating effects on fish farming especially in intensive systems, resulting in huge economic losses. The aim of this study was to evaluate whether dietary supplementation with free- or nano-form of curcumin improves growth performance, immune status, and heat stress resistance in Nile tilapia (*Oreochromis niloticus*).

\Seven diets were prepared, six of which contained 3 levels of curcumin nano-sphere (50 (CN50), 100 (CN100) or 200 (CN200) mg kg^{-1}) or free curcumin (50 (C50), 100 (C100) or 200 (C200) mg kg^{-1}), and one diet contained no addition (CON). Fish (13.54±0.32 g) fed the experimental diets for 65 days and then they subjected to the acute heat stress by gradually raising the water temperature from 25 to 40° within 3 h. Results revealed that dietary curcumin has enhanced growth performance with superiority to nano-curcumin over the free form. The optimum growth performance was obtained at CN100 (Fig.1). Heat stress rather than diets increased platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), leukocytes and neutrophils counts while lymphocytes decreased. CN50 and CN100 reduced the activity of liver enzymes (Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) more efficiently than the other diets and C200 gave the highest activity of these enzymes. CN100, CN200, C100 and C200 had higher immunoglobulin (IgM) levels than CN50 while C50 and CON gave the lowest values. C200 showed a higher level of complement C3 and C4 than the other treatments while C50 and CON gave the lowest values (Table 1). CN50 and CN100 have efficiently eliminated cortisol levels compared to the other treatments. All curcumin-supplemented diets, except for C200, produced lower glucose values compared to CON. After heat stress, ALT, AST, IgM, C3, C4, cortisol and glucose have been increased. Thus, curcumin nano-sphere has better ability than its free form to increase the ability of farmed fish to withstand heat stress, enhance growth rates and thus increase fish productivity.

Table 1. Effects of experimental diets on IgM, C3 and C4 of Nile tilapia

		CON	CN50	CN100	CN200	C50	C100	C200	SDM
25°C	IgM	0.82	1.30	1.88	1.87	1.11	1.84	1.72	0.43
	C3	1.30	2.16	2.72	2.49	1.26	2.20	2.28	0.59
	C4	2.47	3.56	3.66	4.02	3.23	3.61	3.80	0.59
40°C	IgM	1.88	2.34	2.92	2.89	1.95	2.87	2.79	0.46
	C3	3.42	4.65	4.80	4.45	4.13	5.47	5.59	0.52
	C4	5.23	6.83	6.20	7.13	4.83	5.70	7.71	1.05

SDM, mean standard deviation

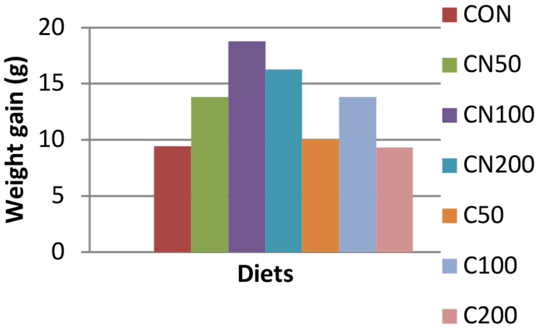

Fig 1. Effect of experimental diets on WG of Nile tilapia

IMMUNOMODULATORY LECTIN-LIKE PEPTIDES FOR FISH ERYTHROCYTES-TARGETING AS POTENTIAL ANTIVIRAL DRUG DELIVERY PLATFORMS

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Introduction

One of the challenges of science in disease prevention is optimizing drug and vaccine delivery. Until now, many strategies have been employed in this sector, but most are quite complex and labile. To overcome these limitations, great efforts are directed to coupling drugs to carriers, either of natural or synthetic origin. Among the most studied cell carriers are antigen-presenting cells (APCs), however, red blood cells (RBCs) are positioned as attractive carriers in drug delivery due to their abundance and availability in the body. Furthermore, fish RBCs have a nucleus and have been shown to have a strong involvement in modulating the immune response. In this study, we evaluated the binding of three peptides to rainbow trout RBCs, two lectin-like peptides and another derived from Plasmodium falciparum membrane protein, in order to take advantage of this peptide-RBCs binding to generate tools to improve the specificity, efficacy, immunostimulatory effect, and safety of the antiviral therapeutic or prophylactic administration systems currently used.

Material and Methods

In order to evaluate the binding of the peptides to RBCs, we carried out a dose-response assay using a concentration range of Rhd-labeled peptides. The binding of the peptides was evaluated by means of flow cytometry. Moreover, an *in silico* evaluation of the peptides' physicochemical descriptors was carried out. Also, we investigated whether the treatment with the three peptides could generate an antiviral immune response in RBCs, *in vitro*, by means of examining the expression profile of some genes related to antiviral activity.

In order to track *in vivo* the binding of the peptides to RBCs, we examined the peripheral blood of rainbow trout individuals injected with the Rhd-labeled peptides, and blood was analyzed by means of flow cytometry and fluorescent microscopy.

Results

Our results demonstrated the binding of peptides to rainbow trout RBCs, with a higher affinity of two of them *in vitro* and *in vivo*. Additionally, peptide-RBC binding was confirmed by confocal microscopy which showed the presence of all the peptides inside the cell. In general, gene expression analysis showed that these peptides were capable of modulating the expression of genes related to the antiviral immune response.

Briefly, our results showed that two of these three peptides bind to rainbow trout RBCs and they are good candidates for linking up to new delivery systems and enhancing the antiviral immune response during treatment.

SOFT X-RAY CRYO-TOMOGRAPHY REVEALS THAT ENDOPLASMIC RETICULUM PLAY AN ACTIVE ROLE IN RAINBOW TROUT ERYTHROCYTES ANTIVIRAL IMMUNE RESPONSE

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Introduction

Fish nucleated red blood cells (RBCs) play an active role in the homeostasis of the fish immune system as they can modulate the expression of different proteins in response to various stimuli, including the attack of a virus. These functions include the activation of pathogen elimination mechanisms and the activation of autophagy and genes related to the immune response. Furthermore, when rainbow trout RBCs are exposed to the viral haemorrhagic septicaemia virus (VHSV), in addition to generating an antiviral immune response, these cells appear to undergo intracellular changes and, in the end, RBCs halt VHSV infection. In this work, cryo soft X-ray tomography (cryo-SXT) has been used to elucidate the structural changes induced in rainbow trout red blood cells exposed to UV-inactivated VHSV and we have focused on studying the involvement of endoplasmic reticulum stress in the antiviral response in RBCs.

Material and methods

First, rainbow trout RBCs were purified by ficoll density gradient centrifugation. Then, RBCs were exposed to UV-inactivated VHSV and samples under cryogenic conditions were transferred to Mistral beamline (ALBA light source) at ALBA synchrotron, to obtain the X-ray tomography data sets.

In addition, time course of ER stress-related genes expression was carried out on RBCs and rainbow trout gonad-2 cell line (RTG-2) exposed to VHSV or UV-inactivated VHSV. Also, RBCs and RTG-2 cells were incubated with a stress inhibitor both post-exposure and pre-exposure to VHSV. ER-stress was evaluated by means of immunofluorescence and RT-qPCR.

Results and conclusions

Cryo-SXT images showed remarkable cellular modifications. One of them was the increment of the endoplasmic reticulum (ER) in RBCs exposed to UV-inactivated VHSV, which was related to the stress of the ER and the activation of the unfolded protein response (UPR) pathway. Another one was the production of extracellular vesicles (EVs) in RBCs exposed to UV-inactivated VHSV.

On the other hand, the expression of ER stress-related genes was increased in RBCs and RTG-2 cell line in response to UV-inactivated VHSV and there was a direct effect between ER stress inhibition and VHSV replication levels in both cell types.

These results open a new line of investigation related to the role of ER stress, UPR, and EVs in the immune response induced by RBCs in response to viral vaccines.

DAILY RHYTHMS IN THE STRESS RESPONSE OF THE EUROPEAN SEA BASS (*Dicentrarchus labrax*): EFFECTS OF SEASONALITY AND FEEDING TIME

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Introduction

Fish in aquaculture facilities must cope with different stressors like handling that involve air exposure. This operation can elicit a high stress response due to manipulation and a strong oxidative reaction due to air exposure followed by reoxygenation. The stress response starts in the hypothalamus and culminates with cortisol secretion. The antioxidant system responds with a different regulation of its principal factors like catalase (*cat*), superoxide dismutase (*sod1*), glutathione reductase (*gsr*) and glutathione peroxidase (*gsh-px*) (Scandalios et al. 2005). Mitochondria are involved as well in maintaining the balance between the oxidative and antioxidative processes and encode for genes considered as mitochondrial biomarkers for fish welfare like uncoupling protein (*ucp*), peroxiredoxins (*prdx*) and cytochrome c oxidase 4 (*coxIV*). *Fish* are subjected to environmental variables that act as *zeitgebers* or synchronizing factors in the natural environment. These *zeitgebers* follow a predictable pattern based on cyclicity. They have promoted the development of the circadian system based on input signals, pacemakers, and output pathways, coordinated by a self-sustainable clock at the molecular level that relies on positive and negative feedback (Pando and Sassone-Corsi 2002). A molecular connection between the circadian system and physiological stress response has been proved in several species (Cowan et al. 2017), with a species-specific pattern, but nothing is known about this subject in the European sea bass (*Dicentrarchus labrax*). Moreover, the components of the antioxidant system have been described in fish, but only a few studies focused on the time-dependent response of the genes involved (Vera et al. 2014). Additionally, seasonal variations in basal cortisol levels have been described in the sea bass (Planas et al. 1990), making it reasonable that other components of the stress response could display differences based on the season of the year. This study aimed to clarify if the European sea bass presents variations in the stress and antioxidant responses to air exposure depending on the time of the day or season of the year. For this purpose, fish were exposed to air for 1 minute in two moments of the year (December and June). Physiological stress indicators in plasma, genes of the hypothalamus-pituitary-interrenal (HPI) axis, genes involved in the oxidative response and mitochondrial oxidative biomarkers were analyzed for this scope.

Materials and method

The European Sea bass specimens (N = 196 for each seasonal trial) were placed into 14 tanks, randomly divided into two groups (CTRL and STRESS - 7 tanks each group) and fed once a day in the mid-light phase (ML). They were kept under natural seasonal conditions of photoperiod and water temperature in an open recirculatory system. Fish were maintained under these conditions for one month prior to sampling in December (10:14 LD; 19,47 ± 0,17 °C) and June (14:10 LD; 22,13 ± 0,13 °C). CTRL and STRESS groups were sacrificed during a 24-h cycle, in 7 sampling points, respectively at ZT 0.5, 4, 7.5, 12, 16, 20 and 24.5 h. Fish from the CTRL group were sampled directly, while fish of the STRESS group were exposed to air for 1 minute and sampled 1 h later. Samples of plasma, liver and hypothalamus were collected.

Results

The plasmatic levels of cortisol, glucose, and lactate displayed a significant increase in the STRESS group in both seasons. Surprisingly, the hypothalamic gene *crh* showed a different pattern, and the stress group had not consistently higher expression levels, while *crh-bp* presented more differences between control and stress. Genes involved in the antioxidant system (*cat, sod1, gsh-px* and *gsr*) analyzed in the liver responded significatively to air exposure in both the season. In these genes, an effect of the season was observed as higher differences between the CTRL and STRESS groups were detected in June. Concerning the mitochondrial biomarkers (*ucp1, prdx3* and *coxIV*), they responded to stress with different patterns depending on the season. In this study, we also investigated the existence of daily rhythm in the parameters analyzed. It was confirmed for most of the physiological parameters analyzed but with significant differences between the control and stress groups and between the seasons studied, December and June. For the genes with a daily rhythm confirmed, the acrophases were predominantly nocturnal, both in December and June. Additionally, the season seems to influence the appearance of a rhythm since a significant part of the biological parameters that displayed rhythms in December seemed to lose it in June.

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Conclusions

In the present study, the physiological response to air exposure was evident, especially in the plasmatic levels of cortisol, glucose, and lactate. In the liver, the response of the antioxidant genes was clear as well. Additionally, the season seems to play an important role since in the antioxidative response is markedly different compare December and June. The study of the daily rhythms revealed that they are present in most of the physiological parameters studied, both in control and stress with a nocturnal acrophases. In this case, again, the contribution of the season is relevant since in June only a few of them continued to display rhythmicity. Taken all together, these data suggest that the stress response is not only species-specific but also time-dependent and influenced by the season of the year.

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HOW FEEDING TIME AFFECTS CLOCK AND EPIGENETIC MECHANISMS IN LIVER AND BRAIN OF EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

In response to an environment based on the 24-hour light-dark (LD) cycle, fish have evolved circadian systems based on a 24-hour periodicity and synchronized by different *zeitgebers*, like the photoperiod and feeding. At the molecular level, a circadian rhythm is created by a self-sustainable molecular clock formed by positive and negative feedback loops. The positive feedback is represented by *clock* and *bmal,* which heterodimer promotes the transcription of *cry* and *per*, also known as the negative feedback since their product will translocate into the nucleus to inhibit the transcription of *clock* and *bmal* (Pando and Sassone-Corsi 2002). The mechanism and the rhythm established at different physiological levels are controlled by the intersection of the positive and negative loop, but the epigenetic mechanisms of deacetylation and methylation are also involved (Reszka & Zienolddiny 2018). In the deacetylation process, *sirt1* plays a crucial role (Nakahata et al. 2009). Concerning methylation, DNA methyltransferases could be involved in controlling the positive loop and *dnmt3* might be responsible for the methylation of *bmal's* promoter (Satou et al. 2013). Moreover, different processes are involved in the erasing of the methylation, pointing out that the contribution to the molecular clock could come from different levels. Additionally, both sirtuins and DNA methyltransferases are linked to nutrient availability (Su et al. 2016), suggesting that feeding time can act on the clock mechanism also through the epigenetic way. This study aimed to understand if feeding time can affect the rhythms of expression of clock genes and genes from the epigenetic machinery in the liver of the European sea bass (*Dicentrarchus labrax*), and a possible connection between the two systems. Additionally, clock genes were also analyzed in the hypothalamus to understand if feeding time affects the central pacemaker.

Materials and method

Fish were kept in an open recirculatory system in a 14:10 L:D cycle and fed with 1% of the fish body weight, once a day at fixed times. To understand how feeding time can affect rhythms of expression of clock genes and genes from the epigenetic machinery, fish were divided into two groups, fed at mid-light (ML) or at mid night (MD) respectively. During the sampling, fish were sacrificed in a 24-h cycle during seven sampling points, respectively at ZT 0.5, 4, 7.5, 12, 16, 20 and 24.5. Samples from hypothalamus and liver were collected for gene expression analysis. In hypothalamus, the analysis focused on the clock genes (*clock, bmal1, per1, per2, cry1* and *cry2*), while in the liver, clock genes and genes of the epigenetic machinery (*sirt1, dnmt1, dnmt3a, gadd45aa, mbd4* and *tet2*), were analyzed.

Results

All the clock genes analyzed in the liver (*clock, bmal1, per1, per2, cry1* and *cry2*) displayed rhythms in the ML group, while only *per2* displayed a rhythm in the MD group. Concerning the epigenetic genes analyzed (*sirt1, dnmt1, dnmt3a, gadd45aa, mbd4* and *tet2*), their rhythm was confirmed in the ML group, while only *dnmt3a* displayed a rhythm also in the MD group. Additionally, all the rhythms found in epigenetic genes had their acrophases during the night. Finally, rhythms were found in the hypothalamus for the clock genes both for ML and MD groups, except *cry2*, where no rhythm was found.

Conclusions

In the liver, rhythms were present in all clock genes analyzed in the ML group but not in the MD group with the exception of *per2*. This suggests that the feeding time has a strong impact since, when fish are fed in MD, the rhythm seems to disappear. Additionally, in most of the genes, the differences between ML and MD gene expression are significant, underlining the fact that the feeding time also influences the levels of transcription. Concerning the deacetylation mechanism, *sirt1* displays rhythm at least in ML. In most of the ZT points, a significant difference between both groups (ML vs. MD) is found in the transcriptional level, which could depend on the availability of NAD+ that it uses as cofactor, and it's related to the energy status. Additionally, its acrophases is similar to the one of *clock*, which counteract its acetylase activity. In the methylation process, *dnmt1* seems to be more affected by the feeding time than *dnmt3a*, suggesting that the maintenance of the pattern of methylation in the cells could be impacted by the energy status. The genes involved in the demethylation process presented rhythms only in ML but not MD, revealing their sensitivity to feeding time. Moreover, differences between ML and MD are present in several ZT points, with the value of MD group usually higher, suggesting an increase of the demethylation potential. In the hypothalamus, most of the clock genes analyzed displayed rhythm both in the ML and MD

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groups, confirming that the feeding time is not the first synchronizer in the central pacemaker. However, comparing ML and MD groups, significant differences are present between the same ZT point, so even if feeding time cannot be the major synchronizer, it can affect at least the expression, especially of the positive loop. To better understand the contribution of the epigenetic process to the clock genes, our research is currently focusing on the analysis of the methylation of *bmal1* promoter.

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HOW FEEDING TIME AFFECTS THE DAILY RHYTHMS OF FOOD INTAKE CONRTOL AND DIGESTIVE FACTORS IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

The regulation of food intake is a complex process where central and peripheral endocrine factors act to stimulate or inhibit feeding. Specifically, the hypothalamus plays a central role in integrating circadian and metabolic information and neuroendocrine signals. Additionally, it produces neuropeptides that can be divided into orexigenic and anorexigenic based on their action on feed intake (Volkoff 2016). As a peripheral organ, the liver is implicated in the regulation of feeding since it produces hormones that give information on the energy status. In addition, the digestive system of fish produces and secretes enzymes that are involved on nutrient digestion. All these factors, hormones and enzymes, are under the control of the circadian system and feeding time in fish (Kulczykowska & Sánchez Vázquez 2010). Moreover, the light-dark cycle represents one of the most important signals that can entrain the hypothalamus and its circadian clock, but for peripheral clocks feeding time seems to exert a significant influence (Montoya et al. 2010). The result is a system where the circadian mechanisms affect the metabolic pathway but at the same time the circadian system is affected by metabolism as well (Delgado et al. 2017). This study aimed to understand how feeding time can affect the daily rhythm of the factors involved in appetite control and digestion in the European sea bass (*Dicentrarchus labrax*).

Materials and method

Fish were kept in an open recirculatory system in a 14:10 L:D cycle and fed with 1% of the fish body weight, once a day at fixed times. To understand the importance of feeding, fish were divided into two groups, one group fed at mid-light (ML) and the other at mid-dark (MD). The sampling was performed in a 24-h cycle with seven sampling points at ZT 0.5, 4, 7.5, 12, 16, 20 and 24.5. Samples from hypothalamus and liver were collected and stored at -80ºC until analysis. Gene expression analysis was performed to evaluate the expression of genes involved in the regulation of feeding both in the hypothalamus (*neuropeptide Y*, *agouti-related protein, orexin* and *melanin-concentrating hormone*) and liver (*trypsinogen2, trypsinogen3, chymotrypsinβlike, carboxypeptidase* and *phospholipase*)

Results

In the hypothalamus, the rhythm was found in the MD group for the expression of *npy*, *agrp* and *orexin*, while no rhythm was found in the expression of *mch*. For all the genes that displayed rhythms, the acrophases were nocturnal. In the liver, *trypsinogen3, chymotrypsin-β-like* and *carboxypeptidase* displayed rhythms in the ML and MD groups, and the acrophases were nocturnal with a shift in the acrophases between ML and MD that varies between 3 and 7 hours. For *trypsinogen2* and *carboxypeptidase*, the only rhythm found was in the ML group, again with nocturnal acrophases.

Conclusions

No rhythm has been confirmed in the hypothalamus for the ML group, but only in the MD group for *npy, agrp* and *orexin*. These data suggest that feeding time can impact the rhythm in the brain, but the fact that ML feeding seems not to affect the hypothalamus in terms of rhythm could suggest that another part of the brain could participate in integrating the signals and displaying the rhythm of some genes analyzed. To clarify this hypothesis, a possible candidate for the analysis in the brain would be the telencephalon, where genes involved in the regulation of food intake are expressed like in the hypothalamus. The analysis revealed a clear rhythm in the liver for the genes analyzed in the ML group, suggesting a synchronization due to feeding time. In the genes where the rhythm is present also in the MD group, a shift in the acrophases is present but does not match the 12 hours of differences between the two feeding time, suggesting both light and feeding time interact to determine the acrophases of the rhythm.

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EFFECTS OF WATER TEMPERATURE ON GROWTH PERFORMANCE, SURVIVAL, AND OXIDATIVE STRESS RESPONSES IN JUVENILE SOUTHERN BLACK DRUM (*Pogonias courbina*)

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Introduction

Temperature has a strong influence on fish metabolism, which is correlated with fish growth and ultimately survival (Boltaña et al., 2017). Temperatures outside the optimum range of the species can induce oxidative stress in fish, a condition caused by the imbalance between prooxidant and antioxidant defenses (Nitz et al., 2020). This work aimed to evaluate the effect of temperature on survival, growth performance, and oxidative stress responses of juvenile Southern black drum *P. courbina*.

Materials and Methods

Juvenile *P. courbina* (450 fish, initial weight 0.58±0.19g) were exposed in triplicate to 23, 26, and 29°C for 25 days. The experiment was carried out in 300 L tanks attached to RAS. Photoperiod was set to 12 h light per day, salinity was maintained at 30±1‰, pH was equal to 8.01±0.1, and nitrogen compounds were within acceptable levels.

Fish were fed four times a day, with a formulated diet containing 55% crude protein and 13% lipids. At the end of the trial, weight and length were measured, six fish from each tank were killed and samples of gill, liver, and muscle were frozen in liquid nitrogen for analysis of total antioxidant capacity against peroxyl radicals (ACAP), lipid peroxidation (TBARS), and protein thiols (P-SH).

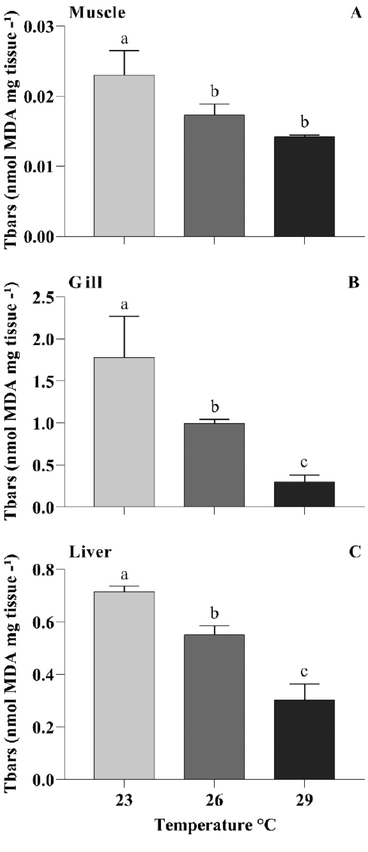

Figure 1 – Lipid peroxidation of muscle, gill, and liver of juvenile *Pogonia courbina*, reared for 25 days at different temperatures.

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Results

Survival was not influenced by temperature, it was above 98% in all treatments. The fastest growing fish were those reared at 29°C. Their final weight (8.94±0.56 g) was significantly higher ($P<0.05$) than fish maintained in any other treatment. Even tough fish reared at 23°C had the lowest SGR (7.38±0.30 % day^{-1}), their final weight was 6.3 times larger than the initial weight. Furthermore, weight gain of fish reared at 29°C was 2.7-fold higher than fish reared at 23°C. The total amount of food consumed was smaller for fish kept at 23°C, and the apparent feed conversion was poorest at the smaller temperature. Regarding responses on oxidative stress parameters on muscle, gill, and liver, cold exposition (23°C) led fish to higher lipid peroxidation (TBARS) in all tissues analysed (Figure 1). The lowest temperature triggered upper regulation on the total antioxidant capacity against peroxyl radicals (ACAP) in gill and liver if compared to 29°C. Fish protein thiols (P-SH) were higher in the liver of organisms kept at 29°C than 23°C.

Discussion and conclusion

The best performance of fish kept at 29°C may be related to the increased total feed intake, coupled to better apparent feed conversion, along with lower lipid and protein damage, plus lower investment in antioxidant capacity. Based on this, we concluded that among the tested temperatures, 29°C is the best temperature for *P. courbina* production

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AN INNOVATIVE APPROACH FOR THE ENVIRONMENTAL ASSESSMENT IN INTEGRATED MULTI-TROPHIC AQUACULTURE: ASTRAL PROJECT

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Introduction

Given the rapidly increase of the aquaculture sector, it is of relevance to provide the industry with solutions that ensure the sustainability and profitability. However, the monitoring of the sustainability of the aquaculture is challenging due to the diverse of production methods that hamper the development of standardized procedures tailored to all systems: from open to close or from extensive to intensive.

ASTRAL is a European project that aims at developing and providing innovative techniques and species combination to improve Integrated Multi-Trophic Aquaculture (IMTA). Specifically, the project is oriented to the full implementation of strategies to increase the sustainability, profitability, and resilience in four IMTA labs: open offshore (Ireland, Scotland), flow-through inshore (South Africa) and recirculation inshore (Brazil) systems, with a regional challenge-based perspective, including fish, mollusc, echinoderm, crustacean, and algae species.

IMTA systems represent a good alternative production method combining the farming of multiple and complementary species from different trophic levels, where extractive species take nutrients otherwise would be wasted affecting the natural environment (Chopin, 2018). Nevertheless, the environmental assessment of IMTA systems is an emerging topic that has not been extensively explored. In this sense it seems of relevance to elaborate on an innovative approach to address, under an environmental perspective, the complexity not only of nutrients recycling routes but also feeding regimes and infrastructure elements.

ASTRAL demonstrates the sustainability of the selected IMTA chains from an economic, social, and environmental perspective, where the latter approach incorporates a life cycle thinking in the assessment, together with the evaluation of practices toward zero waste and the study of circularity attributes associated to the innovative practices.

This work entails the description of how the LCA, circularity and zero waste approaches are employed to provide a comprehensive evaluation that allows the identification of the best IMTA configuration, while provideing recommendations for the development of production methods towards zero waste and consequently more sustainable business models.

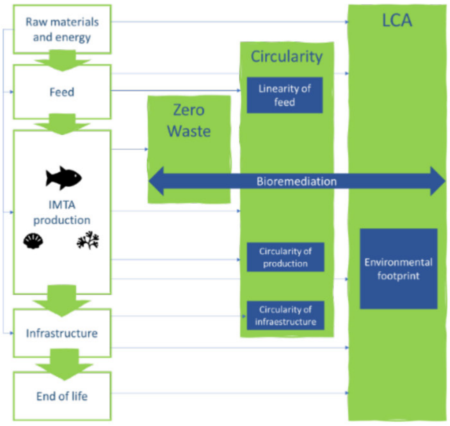

Figure 1. The integrated framework for the environmental assessment of IMTA systems.

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Material and methods

The Zero Waste approach aims at assessing the potential nutrients bioremediation rates in closed IMTA systems, where water and solids are collected and recirculated through the different trophic levels. In open systems, dissolved and suspended solids are released to the environment, so the circularity assessment integrates mass balance analysis to calculate fish emissions, while the nutritional content of extractive species is taken as a proxy to estimate the bioremediation efficiency.

Not limited to the bioremediation, the circularity analysis incorporates a wider perspective where the use of resources and the end of life of materials are considered as well. On the one hand, the development of novel feeds with alternative ingredients sourced from valorisation routes are assessed through circular indicators that inform the linearity of the feed. On the other hand, the infrastructure materials are evaluated in terms of the circularity embedded in plastic and steel elements with recycled components. Both analysis, feed, and production materials, complement the circularity assessment that enables the implementation of an innovative framework to analysis how diverse IMTA systems operate under the principles of the circular economy.

Moreover, and following the recommendations for a more sustainable aquaculture (COM (2021)), the environmental assessment is also materialized with the implementation of a Life Cycle Analysis, that permits the evaluation of the environmental impacts under a cradle to gate perspective. All inputs and outputs needed for the co-production of aquaculture species are gathered and transform into environmental impacts in line with ISO 14040 and 14044 standards. Complementary to zero waste and circularity, the LCA study provides the assessment of eutrophication impact category as a key indicator that represents the efficiency of the IMTA systems in terms of bioremediation (Figure 1).

Conclusions

Having identified the need to harmonise and adapt the methodologies for the aquaculture sector, ASTRAL project brings together the different approaches to create an integrated framework that allow farmers to obtain valuable indicators for decision making to increase the environmental performance of the processes. With a new assessment of circularity and zero waste indicators, ASTRAL develops a LCA study complemented with an IMTA-specific dimension which enables the development of greener business models.

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IFISHIENCI PROJECT: THE ROLE OF NUTRIENT ASSIMILATION IN THE CIRCULAR ECONOMY

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Introduction

Optimization of feeding is a driver to achieve the circular economy in aquaculture. This will enable the enhanced utilization of nutrients and benefits the farming industry through improved production yields with reduced feeding cost, while minimizing the environmental impact of undigested and uneaten feed. Key criteria for the success and continued growth in aquaculture will be to accelerate innovations that can improve sustainable fish feeds, optimize the feeding efficiency, and provide advanced management control for different species in different rearing environments.

The challenge is to find alternative sources at a reasonable cost with the same or similar nutritional value, comparable palatability and digestibility and the potential to substitute fish meal entirely without any negative effect on growth and filet quality. To date, technological developments and commercial applications have mainly focused on algae as a micro-feed ingredient,. Moreover, there is a promise of much higher available quantities of algal biomass in the future (due to its development for use as biofuels), potentially serving the need as a bulk alternative to today's feed ingredients for aquaculture.

Feed, being one of the major costs in fish production is also one of the major constraints for industry growth due to limited sources of sustainable raw materials. Further reduction of fish meal in feed for high value species (e.g. salmon, sea bass) and total replacement in diets of mass produced lower value species (tilapia, carps, African catfish) is necessary to make the world aquaculture more circular and sustainable. The increase of circularity of the production system will be achieved by qualifying methods to obtain new and functional feed ingredients from algae and validating processes for optimal valorisation of waste and sludge from aquaculture production systems.

iFishIENCi is a European project that aims at delivering breakthrough innovations supporting sustainable aquaculture, based on enabling technologies and circular principles, thereby providing the European aquaculture industry with the competitive advantage and growth stimulation needed to be a mover in revolutionizing global efficiency in fish production and meet society's needs for food from the ocean. The project targets circular principles and zero waste by qualifying new and sustainable organic value chains for feeds, and valorisation of by-products. In that sense, in the iFishIENCi project framework, the waste water and sludge collected from the fish growth are used for algae cultivation, reducing those waste streams and growing algae for the production of new feed ingredients. Moreover, the project has actively contributed to the recommendations to incorporate the circularity into the aquaculture sector *(Policy Recommendations For a More Circular Aquaculture*).

Material and methods

In iFishIENCI, the circularity associated to the use of waste streams from aquaculture to produce new feeds are addressed. To do so, available methodologies for circularity assessment are firstly reviewed and later adapted to measure the circularity along the value chain, focusing not only on feed production but also on the farming operation, where the nutrient assimilation plays a relevant role. With regard to feed formulation, ingredients sourced from waste valorisation routes are considered as recycled feedstock. The farming operation is also addressed since the efficiency of feeding is a key element in determining the functionality of the new formulations.

Three main indicators are defined for the circularity assessment of the new feed. Linear flow index (LFI), Material circularity indicator (MCI) and Nitrogen waste indicator (NWi) are the indicators that represents the circularity within the innovative aquaculture systems. The study considers nitrogen, as the principal nutrient involved in the bio recovery cycle.

Material Circularity Indicators of feed is defined in accordance with the Linear Flow Index of nitrogen together with a factor F(X) that is built as a function F of the utility X.

$$MCI = 1 - LFI\ x\ F(X)$$

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The utility reflects the extent to which a product is used to its full capacity. In the context of IFishIENCi, digestibility is addressed in the circularity assessment though the ratio that represents the Nitrogen assimilation efficiency (NE), calculated as follows:

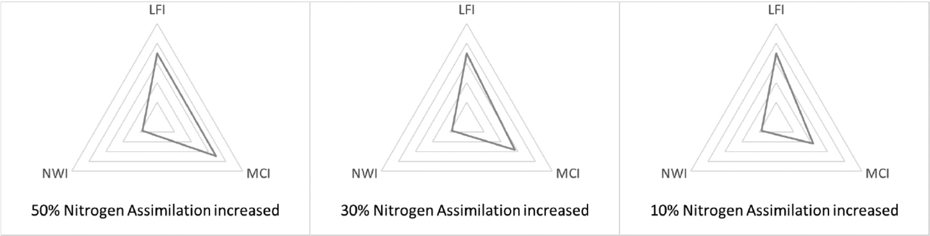
$$N\ assimilation\ efficiency\ (NE) = \frac{N\ assimilated}{N\ ingested}$$

The increase on digestibility, that means the assimilation of nitrogen in feed, results in a more efficient use of any resources that take a linear path in the material flow. Therefore, the higher NE, the higher utility. It is calculated as the ratio between the NE of the new feed formulation obtained in the project in comparison with the NE average of the industry for conventional feeds and not-improved feeding systems.

In the example below, MCI is 58,93% higher when increasing nitrogen assimilation by 50%; MCI is 35,36% higher when increasing nitrogen assimilation by 30%; MCI is 15,69% higher when increasing nitrogen assimilation by 10%. Therefore, notable variations are observed when applying the nutrient assimilation principle to the method and taking different NE ratios when performing the calculations, MCI increases value when the assimilation of nutrients increases.

Conclusions

Improving digestibility, nutrient excretion is minimized, increasing the circularity associated to the feeding systems. Moreover, digestibility and sustainability are linked under the circularity perspective as nutrients are used in a more efficient way, facilitating the incorporation of the circular economy principles in the feed manufacturing process and farming operation.



50% Nitrogen Assimilation increased

30% Nitrogen Assimilation increased

10% Nitrogen Assimilation increased

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Policy Recommendations For a More Circular Aquaculture: available in http://ifishienci.eu/wp-content/uploads/2022/03/IfishcIENCi_Policydoc_Jan-2022Final.pdf

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NATURAL ANTIMICROBIALS FOR AQUACULTURE

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Introduction

Aquaculture has become one of the most important practices for the food industry. Management, health, water quality and pathogen control are determining factors for the success of aquaculture. The search for alternatives for crop improvement and protection against pathogens is of utmost importance to avoid production losses and use of antibiotics. In traditional medicine, plants have been used ancestrally for therapeutic purposes, which is why their antimicrobial potential is recognized. The need for surfaces to provide enough plant tissue for crops can be avoided if aquaponics is implemented. Probiotics have proven to be an effective and safe alternative, not only to limit the development of both bacterial and viral pathogens, but also to improve the general performance of aquaculture crops. When live food is administered in culture systems, it will also be affected by the antibacterial additive, so it is necessary to evaluate said effect, hoping that it benefits the growth of live food or, at least, does not modify it, allowing the administered bacteria generate its probiotic effect in the cultivated organism. The aim of this work is to develop an integral proposal for aquaculture systems to improve farming efficiency through eco-friendly and sustainable strategies.

Material and Methods

The inhibitory capacity of bacterial growth and development was evaluated using extracts of *Ocimum basilicum L* (Basil), *Rosmarinus officinalis* (Rosemary) and *Origanum vulgare* (Oregano). For *O. basilicum*, plants in aquaponic system were also tested.

For the preparation of the extracts, the leaves of each plant were used. The extraction methods were: maceration and rest in ethanol (70 and 96%, 24 h and 15 days), infusion. and decoction in distilled water, stripping with steam and resin. For the growth inhibition tests, Gram (-) microorganisms associated with the culture of the American bullfrog (*Lithobates catesbeianus*), white shrimp (*Litopenaeus vannamei*) and oyster (*Crassostrea* sp.), were used, as well as a strain of *Vibrio parahaemolyticus* (from CIBNOR´s collection) to demonstrate the benefit of extracts for aquaculture. Other marine products (macroalgae and sea urchin extract) were evaluated for their antimicrobial potential.

To evaluate the effect of probiotic bacteria on secondary culture organisms for live food, this study assessed *in vitro* interaction between *Bacillus* bacteria and microalgae and their posterior *in vivo* effect on rearing Kumamoto oyster *Crassostrea sikamea*. The probiotic strains *Bacillus licheniformis* (MAt32), *B. subtilis* (MAt43), and *B. subtilis subtilis* (GAtB1) were individually inoculated in triplicate into 250 mL flasks containing 1×10^4 colony forming units (CFU) mL^{-1} of bacteria and 4.5×10^4 cell mL^{-1} of microalgae (*Isochrysis galbana* or *Chaetoceros calcitrans*) to evaluate their growth during a 7-day culture. Single cultures of microalgae or bacilli served as control. Additionally, *C. sikamea* spat was treated for 28 days with four single/combined bacillus treatments in triplicate at a concentration of 1x106 CFU mL-1 as follows: (a) Control, without treatments; (b) Combination of two antibiotics (10 mg L^{-1}); (c) *B. licheniformis*; (d) *B. subtilis*; (e) *B. subtilis subtilis*; (f) mixed bacilli.

Both bacteria and plant extracts were added to white shrimp cultures in experimental treatments. The administration of the extracts in the form of microencapsulates was also evaluated.

Results

The aquaponic system provided sufficient plant material for the extractions at a density of 1:5.3 with *Oreochromis niloticus*. Visually, there were no differences between the plants on land and in the aquaponic system. Inhibition tests *in vitro* (Bauer *et al.*, 1966) showed a higher inhibition halo with the extract obtained by steam dragging. The highest inhibition halo was obtained with oregano extract and to a lesser extent with basil (Table I).

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Table I. Inhibition of bacterial growth (F= frog, Os= oyster and L= shrimp) with B=Basil, Baq=Aquaponic Basil and O=Oregano extracts. Ø data not available, - without halo, + halo <6.2mm, ++ 6.2-10mm, +++ >10mm

		B			Baq			O		
		F	Os	L	R	Os	L	R	Os	L
Infusion		+	Ø	+	-	+	-	-	+	-
Decotion		-	+	-	Ø	Ø	Ø	-	+	-
Ethanol 20	24 h	+	Ø	+	+	+	-	-	Ø	+
	15 d	-	Ø	-	+	+	-	-	Ø	
Ethanol 70	24 h	+	Ø	+	+	+	+	+	Ø	
	15 d	+	+	+	+	+	++	+	Ø	
Ethanol 96	24 h	+	+	+	+	++	-	+	++	
	15 d	+	+	+	+	+	+	+	+	
Steam drag		+	+	+	+	+	-	+++	+++	+
Resin		+	+	++	Ø	Ø	Ø	+	+	+
Resin 50%		+	+	++	Ø	Ø	Ø	+	+	+

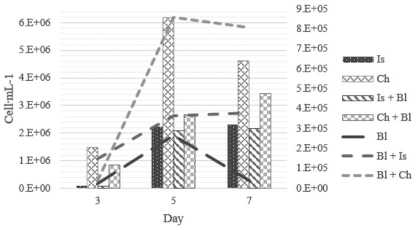

Figure 1.- Microalgae and bacterial growth in co-cultivation. Bars correspond to microalgae and lines to bacterial growth. **Is** = *Isochrisys galvana*, **Ch** = *Chaetoceros calcitrans*, **Is + Bl** = *I. galvana* in co-culture with *Bacillus licheniformis*, **Ch + Bl** = *Ch. calcitrans* in co-culture with *B. licheniformis*, **Bl** = *B. licheniformis*, **Bl + Is** = *B. licheniformis* in co-culture with *I. galvana*, **Bl + Ch** = *B. licheniformis* in co-culture with *Ch. calcitrans*.

The results showed a significantly ($P < 0.05$) increased growth of *Bacillus* strains co-cultured with microalgae while the growth of *I. galbana* co-cultured with bacteria was not reduced significantly ($P > 0.05$) compared with the control group (Fig.1). *C. sikamea* spat treated with *Bacillus* showed significantly ($P < 0.05$) higher growth and survival than the control group. In this study, *C. calcitrans* microalgae were susceptible to the presence of probiotic bacteria. Nonetheless, this reduction in microalgal growth observed *in vitro* increased growth and survival of *C. sikamea* spat exposed to probiotic bacteria compared to spat without probiotics.

To test plant tissues, experimental white shrimp *P. vannamei* cultures were used added with oregano and basil. No significant differences were found in growth with respect to the controls, but a slight difference was observed in terms of survival, decreasing in the treatments due to the toxicity of the plant even mixed with the food, therefore, the addition of the extracts as microencapsulated was evaluated.

Conclusions

With the results obtained, it will be possible to evaluate the inclusion of medicinal plants and probiotic bacteria in the feeding of cultured organisms. The incorporation of plant extracts requires fine-tuning the mode of administration, through microencapsulations. Use of natural additives in aquaculture can contribute to increased growth and survival, and improve the immune response, giving greater resistance to pathogens in a sustainable manner.

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Bacillus SPP. SECONDARY METABOLITES PROTECT ZEBRAFISH FROM INFECTIONS

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Introduction

Aquaculture bacterial outbreaks are responsible for huge annual economic losses worldwide, remaining a major challenge for sustainable aquaculture expansion. Although antibiotics are an important tool for disease treatment, their damaging effects on the environment and public health have led to increased restrictions on their use in aquaculture. Thus, new biological measures are emerging to control the occurrence of bacterial diseases in aquaculture, assuring advanced and integrated health care for humans, animals, and the environment. Over the last years, fish-gut-associated microorganisms have been exploited as an effective approach to face the challenges of modern aquaculture. Among these, *Bacillus* spp. have recognizable biotechnology applications including the production of Natural Antimicrobial Compounds (NACs) that may constitute a barrier against pathogens' proliferation by targeting their growth, biofilm formation, and Quorum-Sensing and the stimulation of the host immune system.

After validation of the *in vitro* bioactivity of three promising *Bacillus* spp. secondary metabolites (1, 2), here we evaluate their potential protective effects and established the best course of treatment using an *in vivo* model when challenged with *Edwardsiella tarda*.

Materials and Methods

Zebrafish experiments and handling were approved by the Animal Welfare Committee of the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), performed by trained scientists (with FELASA category C), and carried out in a registered installation (N16091.UDER), in compliance with the European directive 2010/63/EU for the care and use of laboratory animals.

In this work, we used three Fish isolates (*B. subtilis* FI314, *B. vezelensis* FI436, and *B. pumilus* FI464) previously isolated from the gut of different fish species (*Sparus aurata*, and *Dichentrarchus labrax*) and selected for their *in vitro* bioactivities (1, 2).

Before evaluating the *in vivo* protection, we determined the maximum non-toxic extract concentration (MNTC) of each FI following the Organisation for Economic Co-operation and Development (OECD) Guidelines. Further, we establish the *Edwardsiella tarda* infection model by immersion using 10 dpf zebrafish larvae. Larvae were distributed into 6-well plates containing 10 larvae/well in 5 mL of Egg water and inoculated with $5x10^7$, $1x10^8$, and $3x10^8$ CFUs mL^{-1} of *E. tarda*. After inoculation, larvae were fed, and the plate was incubated at 28 ºC. The *E. tarda* inoculum was kept in the water for 24 h. Cumulative mortalities were registered for 48 h and dead larvae found during the assay were removed.

Finally, to evaluate the best course of treatment for *Bacillus* spp. secondary metabolites, zebrafish larvae were treated with the FI extracts once before mouths' opening, once three days before the bacterial challenge, once one day before the challenge, and every day after the mouths' opening and until the bacterial challenge. Thus, 10 healthy larvae were distributed into each well of a 6-well plate, containing 5 mL of Egg water and treated accordingly with the extracts for 2 h at 28 ºC and then transferred to new 5 mL egg water. The treated 10 dpf larvae were challenged by immersion with *E. tarda*

(Continued on next page)

1×10^8 CFUs mL^{-1}. After inoculation, larvae were fed, and the plate was incubated at 28 ºC for 24 h. Cumulative mortalities were registered between 16 and 48 h and dead larvae were removed and discarded. Control groups were included: (i) non-treated larvae inoculated with *E. tarda*; (ii) non-inoculated larvae; (iii) larvae inoculated with 1xPBS. The experiment was independently performed 3 times.

Survival data were analysed using Kaplan–Meier, and group differences were analysed by the log-rank, using the GraphPad Prism 9 software.

Results

The first step of this work was to determine the maximum non-toxic extract concentration of each FI extract to be employed in protective assays using zebrafish larvae. The results showed that extracts from the three FI induced toxicity in zebrafish when administrated at concentrations above 250 μg mL^{-1}. Secondly, we established the *E. tarda* infection model by immersing 10 dpf zebrafish larvae with three different concentrations of *E. tarda* for 24 h. Larvae exposed to 3×10^8 CFUs mL^{-1} started to show mortality at 10 h post-infection (hpi) and rapidly progressed until 18 hpi, with 100% mortality. In larvae exposed to 1×10^8 CFUs mL^{-1} mortalities began at 17 hpi and progressed through time, reaching ~60% at 24 hpi. From these results, we selected 1×10^8 CFUs *E. tarda* mL^{-1} as the bacterial concentration for the challenge experiment.

Finally, the protective effects and best course of treatment of the FI metabolites were determined using the *E. tarda* infection model and the FI extract maximum non-toxic concentration. Larvae treated once before the mouth's opening and once one day before the challenge did not exhibit any effects on zebrafish survival upon bacterial challenge, when compared to non-treated larvae challenge with *E. tarda*. Zebrafish larvae treated every day with the FI metabolites after the mouth's opening until the bacterial challenge induced higher mortalities when compared to the non-treated and challenge larvae ($p<0.01$), causing 100% mortality after 24h of infection, which might indicate a potential overstimulation of zebrafish's innate immune system. On the other hand, when zebrafish larvae were treated only once three days before the bacterial challenge, we observed a significant increase in larvae survival rate up to 50%, when compared to the non-treated zebrafish larvae infected with *E. tarda* ($p<0.001$).

Conclusions

Taken together, our experiments revealed that the three promising fish-gut *Bacillus* spp. produce extracellular molecules that protect zebrafish larvae from *E. tarda* infection only when administrated once three days before the bacterial challenge. Thus, the metabolites produced by FI314 (*B. subtilis*), FI436 (*B. vezelensis*) and FI464 (*B. pumilus*) may be promising tools for disease control in aquaculture.

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DOMESTICATION AND WELFARE IN FARMED FISH

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The domestication of fish species is still in its early stages when compared to terrestrial animals. Although the gap between the time domestication has been underway in terrestrial farm animals and in farmed fish is enormous, there has been a considerable effort towards fish domestication in recent times (Duarte et al 2007). However, the main component in the domestication process is the generation interval (i.e. the average age of the parent animals at the birth of their offspring—note that this is *not* the age at maturity). A comparison between land animals and fish may be found in Figure 1.

Comparison between the generation intervals in terrestrial farm animals (white bars) and fishes (grey bars).

Generation intervals may be in the same order of magnitude in terrestrial animals and fish, but they vary immensely for different species in aquaculture, adding a biological layer of complexity to the implementation of welfare standards for such a diverse group of animals.

The effects of domestication on welfare of farmed fishes are complex to study because fish differ from livestock in genetics, physiology and behaviour, and experience different sensory worlds. Consequently, empathy with fish and understanding of their needs becomes more problematic than with land animals. Additionally, the acknowledgement and study of mental dimensions of fish existence is very recent (Brown 2015). We discuss that higher levels of domestication in fish do not necessarily correspond to better welfare because (1) artificial selection by the aquaculture industry is mostly focused on production-related traits such as growth, and this selection process may have unknown effects on welfare-related traits; (2) the number of fish species presently farmed (circa 400) is 10-fold higher than land animals, rendering the establishment of general welfare guidelines extremely complicated, even because there is immense diversity among fishes in what regards their welfare needs; (3) the current paradigm of the Five Freedoms guiding welfare is out-dated, was designed for livestock and largely considers animals passive with regards to their environment; and (4) there are still severe knowledge gaps in the biology of farmed fishes, especially in welfare-related traits (Saraiva et al 2019). The implementation and regulation of humane fish farming systems should acknowledge the challenges and specificities of farmed fishes (Saraiva et al 2022), integrating industry, academia, advocacy groups and policy makers in a constructive dialogue.

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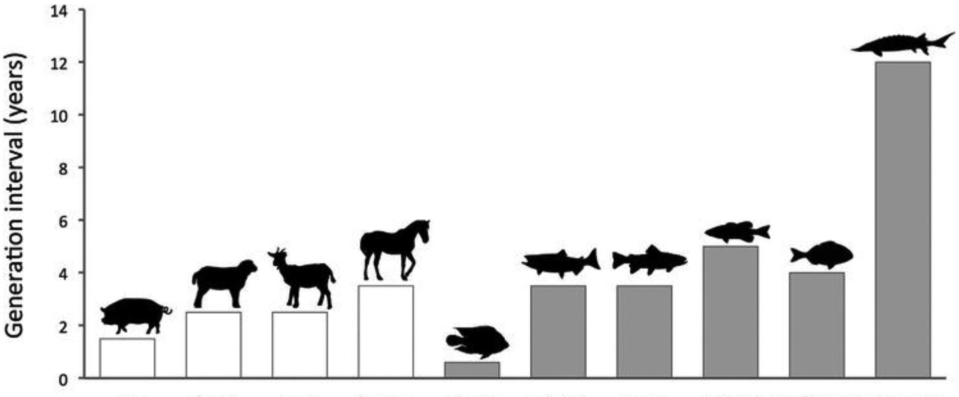

Figure 1.

MAPPING TEMPERATURE STRESS IN RED SEA CUCUMBER *Parastichopus tremulus* USING NON-INVASIVE RESPIROMETRY

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In order to develop IMTA systems with sea cucumbers, a supply of animals in large quantities is needed. The red sea cucumber *Parastichopus* tremulus is found in the northeast Atlantic Ocean, mainly along the Skagerrak and North Sea coast of Norway, Denmark and Sweden, but also Iceland, the Faroe Islands and British Isles, with recorded occurrences down to the Canary Islands. The species can occasionally be found from 18 m down to 4000 m but is most frequent found at 50–500 m on soft sediment bottoms. Fully grown specimens of *P. tremulus* can reach 50 cm in length, 10 cm in width and a wet weight close to 300 g, but the average size and wet weight is around 25 cm and 200 g, respectively Commercial trawling, where sea cucumbers are often caught in large numbers as bycatch, is both destructive to their deep sediment bottom habitat as well as lethal to the animals. By farming red sea cucumber, the development of sustainable aquaculture can proceed, and natural populations be protected from overfishing.

To be able to rear the species on a large scale, it is necessary to know the effect of salinity, ammonium, pH and temperature on its physiological performance in order to be able to optimize the cultivation conditions. We have adapted and developed respirometry equipment for use with sea cucumber *P. tremulus.* We intend to measure the effect of the four mentioned parameters on the animals' oxygen consumption through respirometry studies. Here, we present results on the effect of different temperatures. Oxygen consumption in an organism is an accepted way of non-invasively measuring stress in animals with as little impact as possible.

The focus of our research is to investigate basic physiology and the physiological boundaries regarding environmental biotic and abiotic factors of *P. tremulus.* This will build the knowledge base for developing breeding, hatching and farming guidelines with a focus on animal health and welfare along with productivity for farming new sea cucumber species.

Based on adaptations of several existing protocols for spawning and rearing sea cucumbers, we have also developed a hatching and larval rearing protocol for this species (Schagerström et al 2022). This enabled us to successfully produce the first captivity-bred juveniles of this species in Sweden during 2020.

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EX-SITU AQUACULTURE TRIALS FOR BIOMASS PRODUCTION OF MEDITERRANEAN SPONGES

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Introduction

Marine sponges gaining increasing importance as sources of bioactive compounds for application in medicine, pharmacy, industry and as nutraceuticals. Mediterranean sponge species like *Chondrosia reniformis* and *Axinella polypoides* are of special interest due to their antiviral and antibiotic activities and high amount of *Chondrosia*-collagen (Swatschek et al., 2002). Large amount of sponge biomass is required to obtain a high yield of desired compounds. To avoid overharvesting of natural resources and guarantee a sustainable production, alternative strategies have to be found. The market interest has stimulated research on alternative methods for sponge biomass production such as in situ and ex-situ aquaculture. Although the sponge genus *Axinella* is known to be a source of a variety of metabolites (Menna et al., 2012), so far no attempt has been made to cultivate this species within aquaculture systems. Other than, assessments of in situ mariculture show high growth and survival rates of the marine sponge *C. reniformis* (Gökalp et al. 2019). But it seems to be economically viable production of the respective species is not feasible due to their photophobic characteristics and substrate requirements. *C. reniformis* is being able to de-attach and move around, especially when using common culturing structures, such as pins, lines and net grids (Van Treek et al., 2003). In addition, selecting sites for culturing of this species depending on turbidity and depth respectively sun aversion (Gökalp et al., 2020). Independent of location and weather conditions, ex-situ aquaculture represents a promising alternative as required species-specific conditions can be customized (Nickel and Brümmer, 2003).

Aquaculture trials shall determine optimal conditions for a sustainable sponge biomass production in land-based recirculating aquaculture systems, in special high attachment, survival and growth rates of sponge fragments with triggered compound production.

Materials and methods

Sponge specimen were obtained from either Adriatic or Aegean Sea and subsequently raised in a recirculation aquaculture system at the Alfred Wegener Institute, Bremerhaven, Germany. In various experiments sponges were cut into fragments and placed on different substrate types like artificial substrate (pebbles, quartz sand, cement), clay bowl, clay pot, PPE plate or oyster shell for *C. reniformis* and accordingly clay pot and long line for *A. polypoides*. Tests with different feed types and feeding strategies were conducted. Sponge fragments were fed with either a commercial liquid diet for filter feeders, a self-formulated diet, shrimp-faeces or living microalgae. In parallel approaches sponges were fed once a day or with increased feeding frequency. The effect of nutrient content, temperature, light source (440nm/365+440nm) and current (tank drain bottom/surface) on sponge attachment and survival were examined during the course of the experiment. Fragment growth were measured photographically and the wet weight was determined at the beginning and end of the experiment.

Results

In all experiments the survival rate of sponge (both species) explants were quite high with 80 to 95%. Best survival rates were detected for *C. reniformis* under blue light (440nm) and tank drain at the bottom. Increased algae growth under UV-light (365nm) had no effect on the health status of sponge explants. A further test on temperature increase up to 22°C to enhance metabolism and growth resulted in soaring death rates. Fragments of *C. reniformis* attached easily to any of the substrate types, whereas *A. polypoides* did not attach at all to either clay pot or long line. Statistical tests revealed no significant differences for survival, attachment and growth rates between the different feed or substrate types for both species. But on average increased feeding frequency led to a higher collagen content in *Chondrosia*-fragments fed with self-formulated diet. In general, growth varied greatly among fragments (-35 to 135%).

(Continued on next page)

Discussion and conclusion

To avoid sudden death of sponge specimen short after arrival at the aquaculture facility, efficient transportation conditions as high water volume and cooling are essential. Running-in of culture tanks with water retreatment processes need to give stable nutrient values. Fluctuation of ammonia- and nitrite-contents in the culture tanks has a negative impact on sponge health status. A land-based cultivation of any sponge species could not be implemented yet due to sponge sensitivity to transportation and high demands on aquaculture conditions (Nickel and Brümmer, 2003). Water temperature varies from 17 to 25°C in native environments. Here is a tendency of soaring death rates of fragments with increasing water temperature in connection with unfavourable water parameters. In addition, for reactivation of metabolism and intake of food, sponge fragments need to reorganise their body structure from fragmentation and physical adapt to present flow patterns in the culture tank. Subsequently, any movement of fragments during the experiment has a negative impact on the fitness and formation of a new pumping system. Hence, measurements of growth gives only two values, start and end of experiment.

As sponges are effective active filter feeders with a filtration rate per day of 24,000l/kg sponge biomass (Henchel et al., 2002), optimal feed supply is essential, but in addition high loads of feed can lead to blockage of the ostia whereas low feed supply in starving of sponge fragments (Wilkinson, 1983). It can be assumed that better survival rates are achieved with a special current setting, because the drain at the bottom ensures improved homogeneity of the water body in the tank and thus feed materials are better distributed and waste particles are moved more easily away.

In summary, first attempts to a successful land-based production of sponge biomass have been made, feeding, attachment, survival rates and fitness give reliable values and can be replicated. But for enhanced sponge biomass production there is still further research needed to get more information about the species-specific growth needs and characteristics of sponges.

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IMPACT OF DIFFERENT CULTIVATION CONDITIONS ON THE COLLAGEN LEVEL OF THE MARINE SPONGE CHONDROSIA RENIFORMIS

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Background

Collagen plays a major role in healthcare and well-being and has many potential applications such as in drug delivery, cosmetics and food industries. Thus, the market for collagen is growing rapidly worldwide. Marine sponges are a highly valuable source of marine collagen. Among them, *Chondrosia reniformis* is of particular interest due to the high concentration (> 30%) of type I collagen in the sponge biomass (Ghosh et al. 2019) and the absence of any inorganic components (spicules).

A major constraint regarding the industrial application of sponge collagen is the sufficient supply of sponge biomass. Promising efforts have been made in the mariculture of *Chondrosia reniformis* targeting sustainable production of sponge biomass needed for collagen production (Gökalp et al, 2020; Orel et al, 2021). Land-based aquaculture represents an interesting alternative for the culture of marine sponges, as it allows species-specific conditions and, as a result, maximized collagen production.

Materials and methods

C. reniformis were obtained from the sampling area in the Mediterranean Sea near Ancona/Italy and subsequently reared in a recirculation aquaculture system at the Centre for Aquaculture Research, Bremerhaven/Germany. In different approaches, sponge specimens were cut and sponge fragments were allowed to attach to different substrates (oyster shells, concrete, PVC plates, clay pots and clay plates). In addition, different feeding regimes (feeding duration, feeding frequency) and different food (shrimp faeces, living algae, commercial food for filter feeders, self-designed powder feed) were tested. Sponge collagen was isolated (Swatschek et al, 2002) and collagen content was determined.

Results

The collagen content of *Chondrosia reniformis* was not significantly affected by the substrate they grew on. While *Chondrosia* attached to PVC plates had a mean collagen content of 22%, cultivation on a clay pot and a combination of a clay pot and PVC plate resulted in mean collagen contents of 20% and 24%, respectively. *Chondrosia* growing on clay plates, however, showed a collagen content ranging from 23% to 37% in different experimental approaches.

The diet of the sponges had a significant effect on the collagen content of *Chondrosia*. Sponges fed with shrimp faeces or a combination of shrimp faeces and living algae had a collagen content of 22% and 23%, while feeding them with a self-designed powder feed resulted in 33% and 39% in different experimental approaches. However, food quantity played a significant role to reveal this effect.

Conclusion

Land-based aquaculture of *Chondrosia reniformis* is a promising approach to grow marine sponges and increase their collagen content. In our studies, we found collagen contents of up to 80% in some specimens. This is a clear indication that a systematic increase in the amount of sponge collagen in a closed recirculating aquaculture system is possible. Further studies need to focus on the specific requirements that trigger collagen production in marine sponges.

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SEX REVERSAL TRIALS ON WALLEYE (*Sander vitreus*) AN EXAMPLE OF INTERAGENCYAQUACULTURE COOPERATION

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Introduction

Development of All-female Walleye broodstocks could improve angler satisfaction in Midwestern U.S. states while creation of a YY Male Broodstock could assist with exotic Walleye eradication in Western states *via* the Trojan Y Chromosome of YY Male approach.

Material and Methods

During this pilot study effort, we fed fry feed top-coated with either Estradiol (E2) or Methyltestosterone (MT) at the Rathbun and Meade Hatcheries in Iowa and Kansas. Fish were killed by anesthetic overdose

Results

At 171-176 dph, 255 and 181 fish were euthanized and necropsied from the Meade and Rathbun facilities, respectively. Phenoypic sex ratios for control groups were 53.1% Female, 43.8% Male (1 unknown) from Meade and 57.4% Female and 41% Male (1 unknown) at Rathbun. Preliminary phenotypic sex calls suggest successful recipe development for sex-reversal in both directions. The proportion designated phenotypic or intermediate females in the three E2 treatment trials at 171-176 dph ranged from 96.3 to 98.4%. The number of fish designated as either males or presumed males in the two MT trials at that time were 94.0 and 98.4%. Remaining study fish from both hatchery facilities were subsequently transferred to the Milford Hatchery in Kansas to be reared further to confirm the above sex ratios. At 332dph, a total of 32, 29 and 35 surviving fish from three E2 treatment groups were killed and examined histologically yielding 100% female sex ratios for all three groups (Table 1). The appearance of histological samples was quite clear-cut with readily observed oocytes (Figure 1). Based on both the early phenotypic and the subsequent confirmatory histological results, the study yielded two recipes for highly successful feminization of male walleye. At two years of age, the MT80 and MT100 MT groups were 75 and 80% male based on necropsy, though these results were hindered by small sample size.

TABLE 1: Results of histological examinations from three treatment groups at 332 dph in the estradiol trials at two hatchery facilities

Hatchery	Treatment	Male	Female
Rathbun IA	Control	16	23
	E2 84	0	32
	E2 100	0	29
Meade KS	Control	5	5
	E2 84	0	35

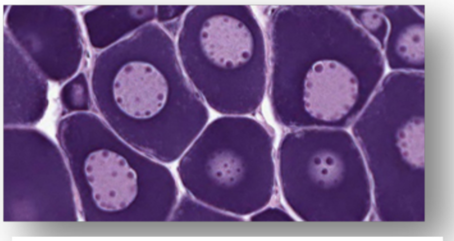

Figure 1. Typical oocyte appearance in 100% of E2 treated Walleye in all three treatment groups.

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Discussion

Based on both the early phenotypic and the subsequent confirmatory histological results, the study yielded two recipes for successful feminization of male walleye. The masculinization results were less clear-cut. However, subsequent full-scale studies yielded highly effective sex reversal protocols for both feminization of male Walleye in North Dakota and masculinization of females in Iowa. We conclude that combining existing fish culture and sex reversal experience from multiple agencies/entities is an efficient approach for development of desirable monosex broodstocks for both commercial aquaculture settings and fisheries management.

FROM AQUACULTURE TO AQUACULTURE: PRODUCTION OF THE FISH FEED ADDITIVE ASTAXANTHIN AND OTHER VALUABLE CAROTENOIDS FROM AQUACULTURE SIDESTREAMS IN *Corynebacterium glutamicum*

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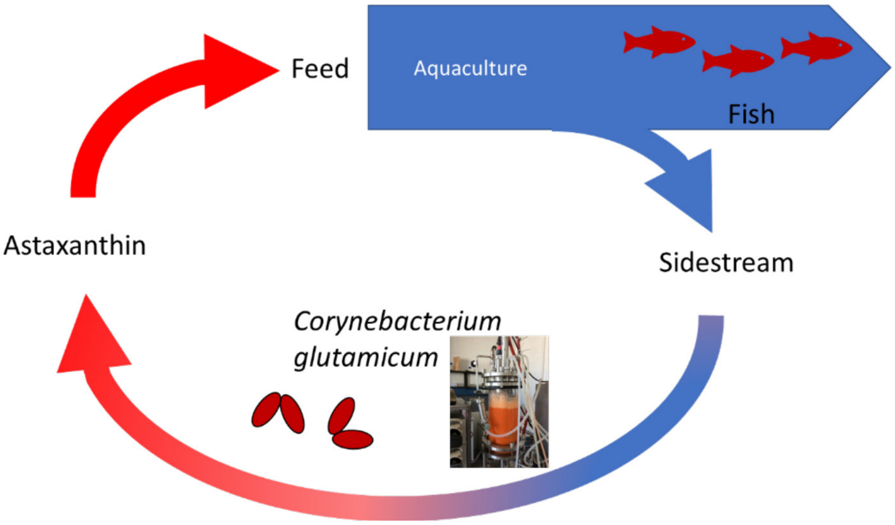
Circular economy holds great potential to minimize the use of finite resources and reduce waste formation by the creation of closed-loop systems. Turning biotechnological production chains into sustainable, circular processes offers the possibility to contribute to climate protection and global waste reduction.

Aquaculture generates nutritious side streams from residual fish feed and fish excrements that may be used for bacterial fermentation processes. Fermentation processes are established in the biotechnological industry creating value from products such as food and feed amino acids or non-animal protein.

Corynebacterium glutamicum is used in the million-ton-scale fermentative amino acid production (Wendisch, 2020), but has also been engineered for production of functionalized amines, diamines, organic acids, alcohols or isoprenoids (Mindt et al., 2020). C. glutamicum naturally synthesizes the yellow C50 carotenoid decaprenoxanthin and was engineered for the production of the red C40 carotenoid astaxanthin (Henke et al., 2016; Henke & Wendisch, 2019). Furthermore, it was engineered for the production of lycopene and β-Carotene (Henke et al., 2017), and the C50 carotenoids bisanhydrobacterioruberin (BABR) (Taniguchi et al., 2017), 2,2′-bis-(4-hydroxy-3-methylbut-2-enyl)-β,β-carotene (C.p. 450) and Sarcinaxanthin (Henke et al., 2017). Carotenoids exhibit antioxidative and ROS quenching properties. They are used as supplements for animal feed in salmonid and shrimp aquaculture, in poultry farming and as food colorants. C. glutamicum grows on a number of carbohydrates and has been engineered to utilize several alternative growth substrates to enable a flexible feedstock concept (Wendisch et al., 2016). C. glutamicum is a very suitable production host for the circular bioeconomy as large scale processes are established and can be scaled and due to its flexibility regarding utilization of various feedstocks and the ease of metabolic engineering towards a broad spectrum of target products.



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In this study, we aimed to utilize an aquaculture side stream (AQ) for production of astaxanthin and other valuable carotenoids. Previously, we have engineered *C. glutamicum* for overproduction of astaxanthin (Henke et al., 2016; Henke et al., 2018; Henke & Wendisch, 2019). Here, we studied if *C. glutamicum* can utilize components present in AQ. First, AQ did not inhibit growth of *C. glutamicum.* Second, AQ could replace a number of components of the mineral salts medium used to support growth of *C. glutamicum*, e.g., phosphorus, calcium, chloride, magnesium and sulfate ions as well as biotin and trace elements. Third, we could show that the standard growth medium supplemented with AQ even enhanced production of astaxanthin. Fourth, the AQ improved astaxanthin production was scaled-up to 2 L bioreactor fermentations. Fifth, this strategy was transferrable and we showed that supplementation with AQ also improved production of the native lycopene and decaprenoxanthin as well as the non-native carotenoids such as β-carotene, zeaxanthin, canthaxanthin, BABR, C.p.450 and sarcinaxanthin.

Thus, we have gained a proof-of-principle for production of astaxanthin from aquaculture sidestreams for use as feed additive in aquaculture, e.g. of salmonids or shrimp. We will discuss the potential and limitations of this aquaculture based approach to circular economy.

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MODELLING THE IMPACT OF SEAWEED CULTIVATION ON THE MARINE PROTIST COMMUNITY

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Introduction

To transition towards a bio-based society, there are efforts to increase seaweed cultivation in the North Sea, especially within areas of offshore windfarms. Seaweed takes up nutrients during winter, grows during spring and is harvested in early summer. Should seaweed cultivation increase on a large scale, the additional uptake of nutrients through seaweed could decrease the availability of nutrients needed for the growth of protists.

Protists are unicellular eukaryotes and they form the base of aquatic food webs. Protist communities consist of phytoplankton, mixoplankton and protozooplankton. Phytoplankton perform photosynthesis and use dissolved nutrients to grow, while protozooplankton acquire nutrition from prey via phagotrophy. Mixoplankton combine photosynthesis with phagotrophy, so mixoplankton can access nutrient and carbon sources that phytoplankton and protozooplankton cannot.

As phytoplankton, mixoplankton and protozooplankton employ different trophic strategies, the additional uptake of nutrients through seaweed could impact the trophic structure of protist communities. A decreased availability of dissolved inorganic nutrients could favor mixoplankton as they do not rely solely on dissolved inorganic nutrients as their source of nutrients.

Material and Methods

To gain insight into the possible impacts of seaweed cultivation on the protist community, a 3D numerical model of the North Sea was modified to simulate the growth of seaweed as well as the growth of phytoplankton, protozooplankton and mixoplankton. Figure 1 illustrates the conceptual ecological model that was applied. Two scenarios were run with (seaweed scenario) and without (reference scenario) taking the growth of seaweed into account. For the seaweed scenario, seaweed was seeded in 25% of all designated offshore windfarms in the Dutch North Sea (i.e. 790 km^2). The two model scenario simulations were run for 12 months from September 2016 to September 2017 and thus covered one growth period of seaweed cultivation.

The reference scenario was quantitatively compared to in-situ measurements of nutrient concentrations and biomass of the protist community. Then, the reference scenario and the seaweed scenario were compared to each other in terms of nutrient concentrations and biomass of phytoplankton, protozooplankton and mixoplankton. Furthermore, the trophic composition of the protist community resulting from the seaweed scenario was qualitatively compared to mesocosm experiments.

Results

Preliminary model results show that the reference scenario manages to capture the timing and magnitude of the nutrient and chlorophyll peak concentrations well compared to in-situ observations. A preliminary comparison between the reference and seaweed scenario show a relative decrease of dissolved inorganic nitrogen and phosphate concentrations as well as a relative shift of the trophic composition of the protist community towards diatoms and mixoplankton. Thus, seaweed aquaculture has the potential to change the trophic composition of protist communities. The comparison of the trophic composition of the protist community resulting from the seaweed scenario to mesocosm experiments is still ongoing research.

Conclusion

The preliminary model results show that seaweed aquaculture has the potential to impact the trophic composition of protist communities. As many harmful algal bloom species are mixoplankton, and diatoms (due to their high nutritional value) are the preferred diet of many higher trophic levels, changes to the trophic composition of protist communities could impact marine ecosystems. In the future, seaweed cultivation will interact with other types of aquaculture, e.g., mussel aquaculture, and potentially with fed aquaculture in an integrated marine trophic aquaculture (IMTA) setting. 3D ecosystem models provide a tool to analyze the effect these scale-dependent anthropogenic changes can have on the trophic composition of protist communities.

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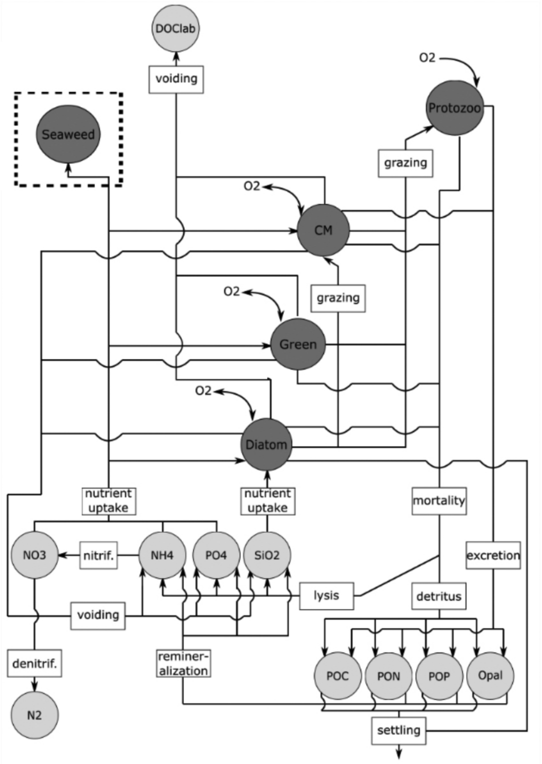

Figure 1 Conceptual ecological model. For the reference scenario, the dashed black box is deactivated. The light grey circles illustrate the abiotic components of the aquatic ecosystem model such as the dissolved inorganic nutrients and the particulate nutrients. The dark grey circles illustrate the biotic compartments of the aquatic ecosystem model such as seaweed, phytoplankton (diatom and green), protozooplankton (protozoo) and mixoplankton (CM).

CREATING DEEPER VALUE FROM LIVE DATA: OXYGEN, TEMPERATURE AND ALGAL BLOOMS

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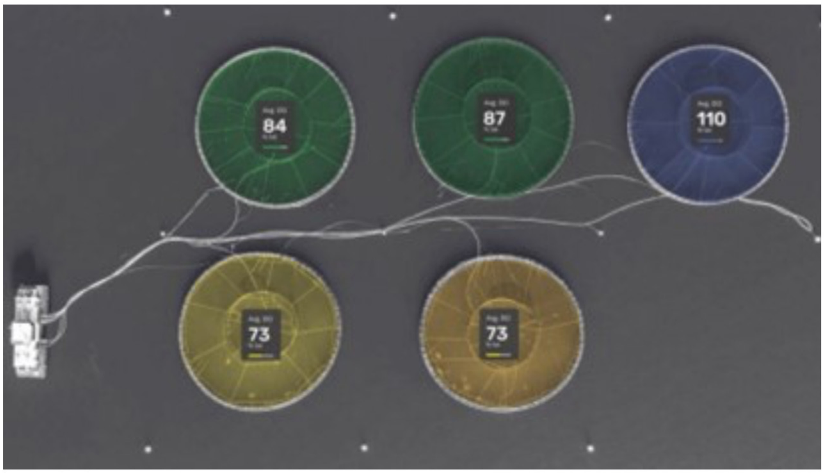
Tools to produce real-time data on aquaculture farms have become increasingly available and cost effective. These sensors enable farmers to make better decisions regarding feeding, aeration, and other operations. As more data becomes available, and systems to utilize those data streams become more sophisticated, farmers are able to create even more value from them.

Two examples will be explored detailing how access to live data creates value for farms. The first will examine in-pen oxygen sensors and how live data can be used to automate control of air diffusers to maintain a healthy environment for fish while minimizing compressor use and reducing fuel consumption. The second example will explore the potential for live data to be analyzed by machine learning algorithms to create forecasts of temperature extremes and algal blooms. These events are stressful for fish and can lead to reduced feed consumption, disease and parasite outbreaks, and mortality. Forecasts can be used to adjust feed schedules and use of air curtains or up-wellers.



EFFECTS OF DIETARY NUCLEOTIDES SUPPLEMENTATION ON PERFORMANCE, PROFITABILITY AND DISEASE RESISTANCE OF *Litopenaeus Vannamei* UNDER INTENSIVE OUTDOOR POND CONDITIONS

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Introduction:

The use of soybean meal (SBM) as replacement for fish meal (FM) in diets for Pacific white shrimp (PWS) has a negative impact on the health of PWS due to its high content in anti-nutritional factors. Dietary nucleotides (N) modulate the immune response and might be able to counteract this effect by enhancing PWS immunity and improving their performance and resistance to diseases. This study was aimed to evaluate the effects of dietary nucleotides supplementation in PWS, *Litopenaeus vannamei*, receiving diets in which fish meal (FM) has been partially replaced by vegetable protein sources.

Methods:

A total of 22,500 shrimp were classified into five study groups and received different diets for 110 days, according to the FM inclusion level and to whether N were added or not: 10FM (Control group; 10% FM), 6FM (6% FM), 10FMN (10% FM; 0.1% N), 8FMN (8% FM; 0.1% N) and 6FMN (6% FM; 0.1% N). Growth performance, body composition, total hemocyte count (THC), lysozyme activity and histopathology of the hepatopancreas were assessed. Organoleptic evaluation and profitability assessment were also performed. In addition, resistance of shrimp to a challenge with *Vibrio harveyi* was studied in shrimps over 5 days after having received diets for 30 days.

Results:

Reducing FM had a negative impact on growth performance (lower body weight and higher FCR) and led to hepatopancreas hemocyte infiltration and enlarged nucleus. Adding N allowed better performance (Table 1), a healthier histomorphological appearance of the hepatopancreas and significantly higher survival rate upon challenge with *Vibrio harveyi* (Figure 1). Moreover, supplementation with nucleotides did not negatively affect the organoleptic parameters and led to a higher profitability.

Conclusions:

Given the observed functional and nutritional benefits of 0.1% N, their nutritional supplementation could be considered to improve performance, profitability, and disease resistance in PSW cultured under intensive outdoor pond conditions.

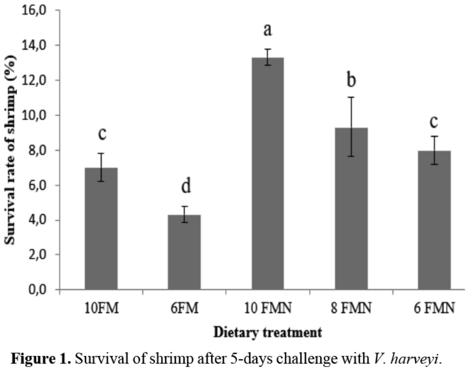

Figure 1. Survival of shrimp after 5-days challenge with *V. harveyi*. Different letters indicate significantly different values ($P<0.05$).

Table 1. Growth performance data. Results in the same columns with different superscript letter are significantly different ($P<0.05$).

Diet	Final Biomass (g)	FBW (g)	Survival pre-challenge (%)	PWG (%)	FCR	TGC	ADG
10FM	7604.0	20.05^a	83.31	1904.66^a	1.36^a	0.05429^a	0.173^a
6FM	7405.5	19.44^b	83.91	1844.15^b	1.40^b	0.05341^b	0.167^b
10FMN	7652.5	20.28^a	83.84	1928.38^a	1.34^a	0.05462^a	0.175^a
8FMN	7521.0	19.98^a	84.67	1897.90^a	1.37^a	0.05419^a	0.173^a
6FMN	7534.0	19.95^a	83.93	1895.46^a	1.37^a	0.05415^a	0.172^a

IMPROVED PERFORMANCE, PROFITABILITY AND IMMUNE FUNCTION IN PACIFIC WHITE SHRIMP FOLLOWING DIETARY NUCLEOTIDE SUPPLEMENTATION UNDER CONTROLLED CONDITIONS IN VIETNAM

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Introduction:

In Vietnam, vegetable protein sources are increasingly being used as replacement for fish meal (FM) in diets for Pacific white shrimp (PWS). This has a negative impact on the health of PWS due to their high content in anti-nutritional factors. Nucleotides (N) modulate the immune response and could counteract this effect by enhancing PWS immunity. This might improve PWS performance and, eventually, optimize profitability. The objective of this feeding trial was to evaluate the effects of dietary N supplementation in PWS, *Litopenaeus vannamei*, under a recirculating aquaculture system (RAS), and receiving diets in which FM has been partially replaced by vegetable protein sources.

Methods:

A total of 1,000 PWS were classified into five study groups and received different diets for 56 days, according to the FM inclusion level and to whether N were added or not: 260FM (Control group; 26% FM), 260FMN (26% FM; 0.1% N), 234FMN (23.4% FM; 0.1% N), 221FMN (22.1% FM; 0.1% N) and 208FMN (20.8% FM; 0.1% N). FM was replaced by vegetable protein sources. Total hemocyte count (THC), lysozyme activity, growth performance, and profitability (return on investment; ROI) were evaluated.

Results:

THC was significantly higher with 260FMN *vs* any other group, and THC in the 234FMN and 221FMN groups (with a 10% and 15% FM reduction, respectively) were also significantly improved compared to the control (regular 26% FM inclusion level) group (Figure 1). N supplementation led to improved performance, achieving better final mean body weight, final biomass, mean weight gain, average daily gain, and feed consumption *vs* 260FM. Several performance parameters were significantly better in the 260FMN group *vs* 234FMN, 221FMN and 208FMN, while this difference was not observed when the 260FM group was compared to the N-supplemented groups with FM reduction (Table 1). ROI improved in all N-supplemented groups (79.5% - 113.3% increase *vs* 260FM).

Conclusions:

Supplementation with 0.1% N for 56 days in PWS under RAS conditions in Vietnam leads to improved performance, immune function, and ROI, allowing FM replacement by vegetable protein sources without having a negative impact on performance.

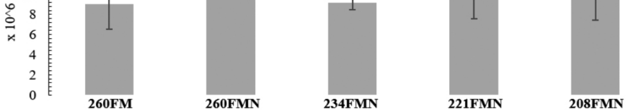

Figure 1. THC in shrimp from the different study groups after 56-day feeding trial. Different letters indicate significantly different values (P<0.05).

Table 1. Growth performance data. Results in the same rows with different letters are significantly different (P<0.05).

	260FM	**260FMN**	**234FMN**	**221FMN**	**208FMN**
Initial mean weight (g)	1.32 ± 0.08a	1.38 ± 0.08a	1.36 ± 0.09a	1.43 ± 0.12a	1.36 ± 0.05a
Initial biomass (g)	52.80 ± 3.35a	55.20 ± 3.35a	54.40 ± 3.58a	57.20 ± 4.82a	54.40 ± 2.19a
Final mean weight (g)	18.87 ± 1.32ab	**19.56** ± 1.12b	17.81 ± 0.85a	18.15 ± 0.93ab	17.61 ± 0.87a
Final biomass (g)	690.32 ± 47.08ab	**711.08** ± 27.62b	665.44 ± 18.52ab	671.66 ± 42.18ab	654.64 ± 25.57a
Mean weight gain (g)	17.55 ± 1.30ab	**18.18** ± 1.08b	16.45 ± 0.82a	16.72 ± 0.82a	16.25 ± 0.88a
ADG (g/day)	0.31 ± 0.02ab	**0.32** ± 0.02b	0.29 ± 0.01a	0.30 ± 0.01a	0.29 ± 0.02a
SGR (%/day)	4.75 ± 0.15b	4.74 ± 0.11b	4.59 ± 0.12ab	4.54 ± 0.07a	4.57 ± 0.12ab
Feed consumption (g)	829.96 ± 19.41b	**845.62** ± 10.68b	844.78 ± 19.51b	844.54 ± 10.16b	810.68 ± 4.16a
FCR	1.31 ± 0.10a	1.29 ± 0.05a	1.38 ± 0.07a	1.38 ± 0.09a	1.35 ± 0.05a

THE EFFECT OF WATER TEMPERATURE AND SUBSTRATE ON THE WARTY SEA CUCUMBER *Neostichopus grammatus* (CLARK) IN A FARMING ENVIRONMENT

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Introduction

The warty sea cucumber, *N. grammatus* (Clark), is a potential aquaculture candidate for use in shore based integrated multi-trophic aquaculture (IMTA) with abalone *Haliotis midae* and several seaweed species at Wild Coast Abalone in the Eastern Cape of South Africa. Theoretically, *N. grammatus*, in co-culture with abalone, could reduce solid waste output from abalone tanks, improve effluent water quality, save farm resources in terms of labour and electricity costs, and produce another high value exportable crop (Qi *et al.,* 2013). The dried sea cucumber product, known as Beche-de-mer or Trepang, is considered a delicacy in several Asian countries and is highly sought after (Purcell *et al.,* 2012). In order to test the viability of co-culturing sea cucumbers with abalone, environmental needs and influencing factors have to be determined for inland breeding purposes.

Materials and Methods

Two environmental parameters (water temperature and substrate) were examined over a two-month period in a farming environment to determine the effects on growth and gonadal development. A Multivariate test of significance and a Repeated Measures Analysis ($p=<0.05$) was performed on the initial (WT 0) and final (WT 1) weights of the sea cucumbers to examine the effect of temperature and substrate on body growth over time. A Univariate test of significance ($p=<0.05$) was performed on the Gonadosomatic Index values to highlight the differences caused by the substrate and temperature on the maturation of the gonads.

Results

Preliminary results are shown in Figures one and two below.

The presence of sand in the sea cucumber tanks had a significant effect on the growth of the sea cucumbers over the two-month trial period. Animals kept at ambient temperature with sand as an additional substrate grew the most over the trial period. Sea cucumbers kept at ambient summer temperature (approx. 19ºC) grew slightly more than animals kept at colder (16ºC) temperatures. The animals that were not provided sand as additional substrate, at any temperature tested, lost body mass.

Captive sea cucumbers kept in tanks with sand had significantly higher GI values than animals kept without sand. Water temperature had a similar effect on gonadal development, although not as significantly.

Discussion

The addition of sand to sea cucumber tanks in farming environments is not common and not always practical. However, the results show that the addition of sand may be necessary when breeding *N. grammatus* sea cucumbers. Addition of sand to the tanks had a significant effect on growth and gonadal development. Animals kept in tanks without sand showed negative growth in terms of body mass and resulted in slower growing gonads. Sand is not only a substrate for the sea cucumbers to stick to, but is also a crucial part of their diet as detritivores (Purcell *et al.,* 2016).

Cold water temperature had a negative effect on body mass compared to ambient water temperature. Animals kept at a colder temperature showed less gonadal development compared to animals kept in ambient temperature water in the presence of sand. The sea cucumbers kept without sand had smaller gonads, with animals kept at ambient temperature showing the least gonadal growth. Animals kept at ambient temperature without sand had higher body masses than animals kept at colder temperatures, however the gonadal development does not produce the same results. This result is anomalous and may be inaccurate due to the short study period. Further research is necessary to conclusively prove what effect these variables may have on growth and gonadal development.

Conclusion

Ingestion of sand as a part of the normal sea cucumber diet is crucial for the inland production of *Neostichopus grammatus* sea cucumbers. Providing sand as a substrate in tanks is somewhat challenging and impractical in a farming environment, but adding sand to formulated sea cucumber feed might prove a possible solution. Furthermore, from a production and export view, *N. grammatus* grows significantly faster in ambient summer water temperatures than in colder water temperatures.

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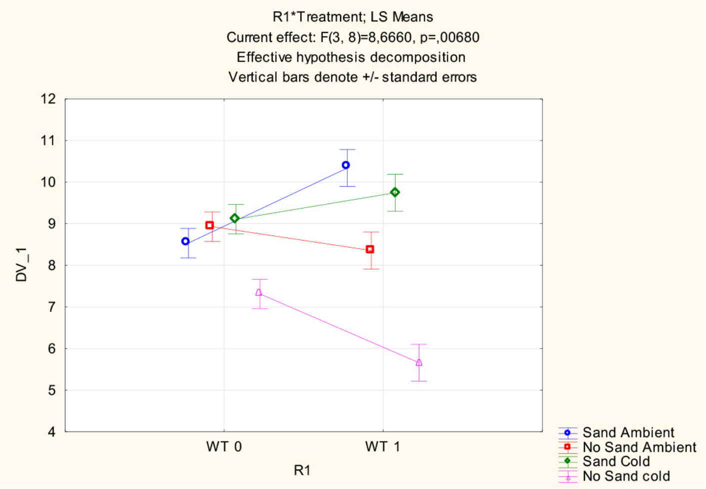

Fig 1: The effect of the four treatments over time (R1) on the total body weights of captive sea cucumbers.

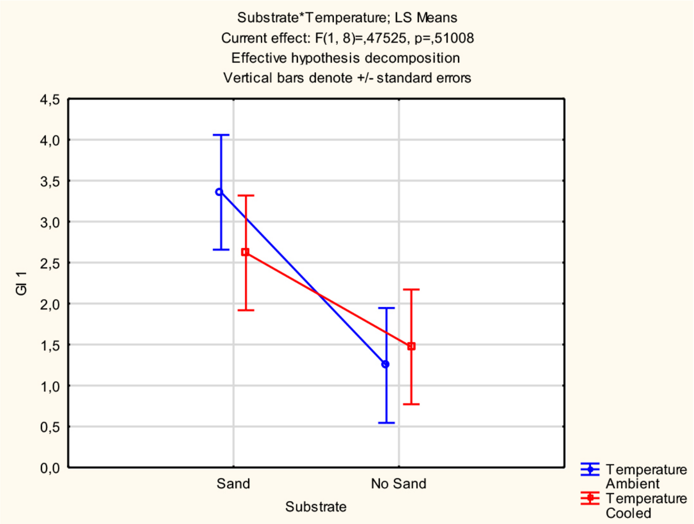

Fig 2: The effect of substrate in sea cucumber tanks on the Gonadosomatic Indices (GI). Gonad weight could only be measured once the experiment had been concluded.

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EFFECT OF PLANT BIOACTIVE MOLECULES ON STRESS AND IMMUNITY OF EUROPEAN PIKEPERCH (*Sander lucioperca*) IN AQUAPONICS

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Introduction

European pikeperch (*Sander lucioperca*) is highly sensitive to environmental and handling stressors in aquaculture (Dalsgaard et al. 2013, Baekelandt et al. 2018)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, limiting the intensification of its farming in recirculating aquaculture. To increase the production of this species sustainably these cultivation modes are of interest (Steinberg et al. 2018) especially to the growing number of small-scale (10 – 20 tons) operations. Plant bioactive compounds can be effective feed ingredients that reduce stress and improve fish growth and immunity. Furthermore, plants also release exudates into the medium surrounding their roots. The influence of such plant metabolites on pikeperch or the performance of an aquaponic system has not been studied to date but could provide an option to improve its cultivation.

The European INTERREG Research project PERCIPONIE aims to develop perciculture in aquaponic systems through improvements on efficiency and fish welfare. One avenue of research is the identification of bioactive compounds emitted by plants cultivated in aquaponics and to test their effects on the growth and immunity of pikeperch. Based on an international consortium of partners, the project brings together stakeholders from research and industry to ensure scientific excellence and applicability of results.

Materials and Methods

The results of the final project stage will be presented. In a hydroponic cultivation experiment, metabolites of plant origin released into the system by different species of vegetables and aromatic plants were identified through untargeted metabolomics analysis. Supported by literature on their chemical properties (size, biological reactivity, solubility etc.) a number of polyphenols were identified. Ongoing experiments test the effect of these molecules on juvenile pikeperch i) in aquaponics systems comparing plant mono-cropping to plant diversity and unplanted control systems and ii) through direct addition of biomolecules to the cultivation water in recirculation systems. Their impact on zootechnical parameters such as growth, stress response and immunity will be evaluated. The results will provide insights into how agro-ecological approaches can be applied to further pikeperch aquaculture.

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CFD ANALYSIS OF WAVE SHIELDING PROPERTIES AND REFRESHMENT RATES IN AN OFFSHORE FISH FARM

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Introduction

In recent years, fish farms are gradually moving from sheltered coastal areas, estuaries and fjords towards offshore locations. Floating fish farm concepts for such exposed environments face a number of design challenges. For instance, extreme storm conditions can challenge the fish welfare as well as the structural integrity of the farm. At the same time, the farm should enable sufficient water refreshment, even in mild conditions, in order to maintain viable oxygen levels and to remove fish waste and bacteria.

A fish farm concept called the Viewpoint Spidercage was recently developed for offshore conditions. The design consists of a fish cage net that is suspended inside a semisubmersible ring that provides support and shelter against wave action (Fig. 1). The design was evaluated in terms of motions, (extreme) wave loads and sloshing behaviour through a combination of wave basin tests and numerical simulations (see van der Plas *et al.*, 2022). The present study follows up on these observations by addressing two additional objectives:

1. To quantify the wave shielding properties inside the fish farm for varying wave periods.
2. To quantify the water refreshment rates inside the fish farm in benign conditions.

Methodology

The shielding and refreshment properties are quantified through 3D computational fluid dynamics (CFD) simulations. The simulations are performed using ComFLOW, which solves the fluid continuity and momentum equations on a staggered grid with a Volume-of-Fluid approach and a piecewise linear interface construction of the free surface (e.g., Veldman et al., 2019). As such, ComFLOW allows the numerical reproduction of highly nonlinear wave interaction with fixed or moving floating structures.

The wave shielding properties were assessed by reproducing an earlier wave basin test in a so-called white noise wave condition, which consists of energy uniformly distributed at periods ranging from 5 to 25 s. Both the incident wave field and the floater's motions in waves were matched and synchronised with the experiments. In the simulations the wave field inside the fish farm was logged and used to calculate the response amplitude operator (RAO_η), which represents the ratio between the wave height inside the farm relative to the undisturbed wave height (i.e., the wave outside the farm). RAO_η is considered a good predictor for the wave transmission into the fish farm because the motion response and wave transmission are expected to be primarily linear for the vast majority of occurring (operational) sea states.

The refreshment properties were studied by keeping the fish farm at fixed position whilst generating different combinations of benign environmental conditions (current speeds up to $V_c = 0.2$ m/s, irregular waves up to significant wave height $H_s = 0.5$ m) at an upstream location. Refreshment rates were quantified by releasing a large number of passive tracer particles inside the fish farm and by Lagrangian-tracking their net removal from the farm due to water exchange at the open bottom of the structure (as illustrated in Fig. 2).

Results

The shielding properties are presented in Fig. 3. The left-hand panel shows the spatially averaged normalized wave height inside the fish farm. For long-period waves (wave period $T > 10.5$ s) RAO_η is close to or exceeds 1 m/m. In this regime the fish farm moves horizontally and vertically with the wave orbital motion and it provides limited shelter. Values over 1 m/m indicate a small amplification of the waves inside the fish farm due to reflections and sloshing. For increasingly shorter waves, which are more predominant in the North Sea, the RAO_η progressively decreases to about 0.4 m/m (i.e., a 60% wave height reduction) for wave periods of about 6 s.

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The right-hand panel in Fig. 3 presents the spatial contour for T = 6.0 s. This period corres-ponds to a standing wave (mode 3) of the wave field inside the moving fish farm. This explains the relatively high RAO_η at x = +/- 15 m from centre (location of anti-nodes) in combination with the low RAO_η at x = 0 m and +/- 30 m. Note that results are presented for regular waves; for more realistic irregular wave conditions less pronounced patterns are expected.

Fig. 4 presents flow field visualizations of the fluid refreshment simulations. The refreshment rates were analysed by studying the number of tracer particles present inside the fish farm over time. The particle counts followed an exponential decay which was used to establish half-life times of the water (i.e., the time required to refresh 50% of the water inside the farm). The following half-life times t_{50} were found:

- Case A. Current only (V_c = 0.1 m/s): t_{50} = 672 s
- Case B. Waves only (H_s = 0.5 m, T_p = 4 s): t_{50} = 1700 s
- Case C. Waves (H_s = 0.5 m, T_p = 4 s) + current (V_c = 0.1 m/s): t_{50} = 1367 s

It follows that the lowest water exchange rates occur for the simulations with only wave forcing (case B). The current contributes importantly to water exchange due to flow separation at the bottom of the ring, leading to a mixing region below the farm (Fig. 4A). In presence of waves, this separation zone becomes more diffuse (Fig. 4C) and the water exchange rates decrease, hence explaining the longer half-life times for case C relative to case A.

It is noted that in field conditions, the incident current will naturally be more turbulent. In addition, motions of the moored fish farm would contribute to fluid mixing and to relative currents between the structure and the water column. As these aspects were not considered, the refreshment rates in the present simulations are expected to be conservative.

Conclusions

CFD simulations were performed to quantify the wave height reduction inside a fish farm and the water refreshment rates due to flow exchange at the open bottom. The results illustrate the potential of high-fidelity numerical methods for design support of floating offshore aquaculture structures.

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GENOTYPE BY ENVIRONMENT INTERACTION FOR PRODUCTIVITY TRAITS OF A HYBRID TILAPIA IN FRESH AND BRACKISH WATER PONDS

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Introduction

Shrimp production is the most important aquaculture industry in Indonesia with the highest contribution to the national income (MMAF, 2018). However, recurrent farming failures and increasing production losses caused by disease outbreaks have hampered the growth of shrimp production, and driven Indonesian shrimp farmers to add tilapia as a second species. The Research Institute for Fish Breeding (RIFB) is developing a fast-growing salinity tolerant tilapia strain. A fresh water nucleus-based program is the simplest strategy to implement but requires knowledge on the extent of genotype by environment (GxE) interaction between fresh and brackish water environments to ensure that genetic improvement in the nucleus transfers to production systems in brackish water. The objectives of this study were: 1) to investigate the presence of GxE between fresh and brackish water ponds, and 2) to estimate genetic parameters for fresh and brackish water environments.

Materials and methods

We produced 91 fish families, and randomly choose 2 groups of 20 fingerlings that we PIT tagged and communally grown in brackish and freshwater ponds (120-147 days). We recorded stocking weight(SW), harvest weight (HW) and standard length (L) at harvest and calculated daily growth coefficient (DGC,(Trọng et al., 2013)) and growth rate in length (GR(L)) from these data. We estimated descriptive statistics using R, and estimated genetic parameters by fitting a series of bivariate animal models in ASReml (Gilmour et al., 2015).

Results

All observed trait of HW, L, DGC and GR (L) at harvest were higher in brackish water and significantly different ($P<0.05$) compared to freshwater (Table 1). The estimates of additive genetic variance (), heritability (h^2) and GCV within freshwater and brackish water for HW, L, DGC and GR (L) are shown in Table 2. Genetic variances and heritability estimates for all traits in freshwater were lower compared to the brackish water, except L. The h^2 estimates for HW, L, DGC, and GR (L) were moderate (0.35 – 0.43). In the freshwater pond, h^2 estimates of HW and L were higher compared to the brackish water while for DGC and GR (L), h^2 estimates were higher in the brackish water compared to the freshwater. The genetic correlation () for all observed trait between freshwater and brackish water were moderate, ranging from 0.65 – 0.74.

Discussion

RIFB has been developing this strain based on selection for own performance (mass selection) for growth and survival in brackish water ponds for 4 generations. However, this breeding scheme has several drawbacks including high mortality of selection candidates during the grow out period in unpredictable salinity condition; security issues; and mortality during transport and adaptation of candidates to the nucleus site in freshwater. Evaluating selection candidates in freshwater ponds and select on sib performance in brackish water could mitigate some of these complications and challenges. However, if G×E interactions are strong, this approach could result in reduced genetic gain due to inaccurate selection of breeding candidates in freshwater (Mulder & Bijma, 2005). In this study, our estimates genetic correlations between fresh and brackish water for HW, SL, DGC and GR(L) were 0.65-0.74 which suggests substantial re-ranking of genotypes between the two environments, suggesting that it is essential to incorporate information from full-sibs in brackish water into the genetic evaluation. Our results also show that HW, L, DGC and GR (L) in brackish water are greater than in freshwater, indicating that the Sukamandi strain has a high salinity tolerance. This is most likely because this strain is a unique composite of Nile tilapia with introgressed salinity tolerance genes from blue tilapia (Yu et al., 2021). The moderate heritabilities for all production-related traits (0.35-0.50) indicate the presence of sufficient additive genetic variance for future selection.

Conclusions

There is GxE interaction between brackish water and freshwater and a breeding program with the brood stock selection process in freshwater should incorporate the sib information of growth performance in brackish water.

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Table 1. Number of observations (N) and mean for stocking weight (SW, g), harvest weight (HW, g), standard length (L, cm), daily growth coefficient (DGC, $g^{1/3}$ /day), growth rate (GR (L),cm/day) and survival rate (S,%) in brackish and freshwater ponds

Trait	Brackish water			Freshwater		
	N	Mean		N	Mean	
		Male	Female		Male	Female
SW	1697	16.8±7.5	16.1±8.6	1711	15.8± 7.3	15.1 ± 8.1
HW	1308	324.7± 61.4*	255.0± 53.8*	1391	261.5 ±49.7*	201.7 ±46.6*
L	1308	19.9±1.3*	18.5±1.3*	1391	18.7±1.2*	17.1±1.3*
DGC	1308	3.5± 0.4*	3.12± 0.4*	1391	2.84±0.4*	2.5±0.4*
GR (L)	1308	0.10±0.01*	0.09±0.01*	1391	0.08±0.02*	0.07±0.01*
S		(77.1)			(81.3)	

*p< 0.05 Student-T test comparing brackish and freshwater ponds

Table 2. Additive genetic variance (σ_A^2), heritability (h^2), genetic (r_g) and phenotypic correlation (r_p) and genetic coefficient of variation (GCV) for HW, L, DGC and GR (L) in brackish water and freshwater ponds

Trait	Brackish water			Freshwater			Correlations	
	σ_A^2	h^2	GCV	σ_A^2	h^2	GCV	r_g	r_p
HW	1048.47	0.35	10.2	790.57	0.38	5.2	0.66	0.24
L	0.48	0.39	3.2	0.53	0.41	2.7	0.73	0.29
DGC	0.47×10^{-01}	0.43	6.5	0.37×10^{-01}	0.42	3.7	0.65	0.28
GR(L)	0.30×10^{-04}	0.42	4.5	0.26×10^{-04}	0.39	2.8	0.74	0.30

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EGG QUALITY INDICATORS IN EUROPEAN EEL AND THEIR RELATION TO DIFFERENT PROTOCOLS FOR VITELLOGENESIS INDUCTION: SPECIFIC mRNA EXPRESSION AND FATTY ACID PROFILES OF EGGS AND EMBRYOS

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Introduction

The obtention of high quality eggs, i.e. eggs that can be successfully fertilized and subsequently develop into a normal embryo, is required for closed-cycle aquaculture (Bobe and Labbé, 2010). While meeting dietary requirements is targeted, egg quality can be influenced by application of assisted reproduction techniques, i.e. the hormonal treatments that are used to stimulate vitellogenesis and oocyte maturation, and/or to induce ovulation (Bobe and Labbé, 2010). These are applied in some fish species that otherwise would not reproduce in captivity, such as European eels (*Anguilla anguilla*), for which vitellogenesis is commonly induced in captivity by chronic administration of salmon (SPE) or carp (CPE) pituitary extracts (Tomkiewicz et al., 2019). In the present contribution, we evaluated different egg quality indicators in batches with high and low larval survival, and the impact of two PE treatment schemes on egg quality parameters in both quality groups using farmed-raised broodstock on an enhanced diet.

Materials and methods

One group of farm-raised females (n = 13) received a weekly intermuscular injection of SPE in a constant dose of 20 mg/kg initial body weight (ibw), during ~15 weeks. A second group (n = 13) received weekly injections of CPE at a biweekly stepwise increasing dose starting at 5 mg/kg ibw and reaching 20 mg/kg ibw from week 10. Samples of unfertilized eggs were collected after stripping for biochemical determinations. Following a standardized gamete production and fertilization protocol, embryos were incubated at optimal temperature and salinity (Tomkiewicz et al., 2019). A sample of 4 hours post fertilization (hpf) embryos was photographed to assess the percentage of irregular cleavages. Samples of unfertilized eggs and embryos at 4 and 24 hpf were taken for molecular analyses. The expression levels of 5 target genes related to cell adhesion (*epcam*, *dcbld1*), stability and structural support (*plec*), and cell division (*cenpk*, *cenpf*) is currently being analysed. Fertilization and hatch success were estimated. Newly hatched larvae were reared in tanks connected to a RAS system. Batches with larvae reaching the first-feeding stage were considered to contain eggs of good quality. Following this criteria, they were categorized in two groups: high quality (n = 12) and low quality (n = 14).

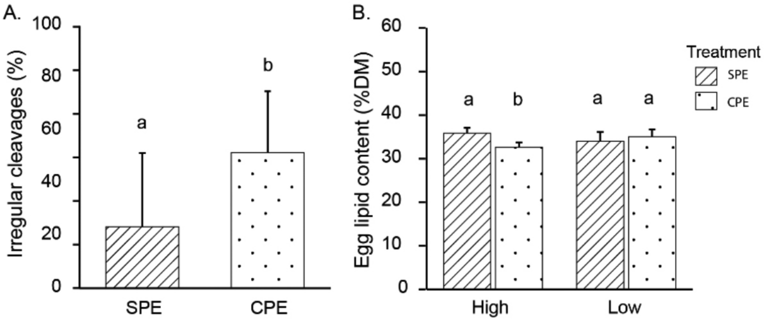

Figure1. Percentage of irregular cleavages of 4 hpf embryos (A) and unfertilized egg lipid content (B) from European eel females subjected to two hormonal treatments.

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Results

Fertilization success was similar for all quality groups and hormonal treatments. Hatch success was also not influenced by the hormonal treatment, but it was 2.4 times higher for the high quality batches. Egg diameter was not influenced by the hormonal treatment and was similar for both egg quality groups. The percentage of irregular cleavages at 4 hpf, on the other hand, was 2 times higher for the CPE than for the SPE treatment, with similar values for both quality groups (Fig. 1A). Total protein content in unfertilized eggs was similar for both hormonal treatments and for both quality groups. Lipid content was significantly influenced by hormonal treatment only in the high quality group, being 1.1 times higher for the batches from SPE treated females (Fig. 1B). The sum of SFA, individual n-3 and n-6 PUFA levels, as well as the sum of PUFA, and the EPA/ARA and DHA/EPA ratios were not influenced by treatment or quality group. However, the levels of C15:0, C16:0, 18:1n9 and sum of MUFA were higher for the CPE treatment than the SPE treatment within the high quality group but similar within the low quality group.

Discussion

Embryos from SPE treated females showed a lower frequency of irregular cleavages, in all quality groups. These results are in agreement with previous studies using constant SPE and CPE doses (Kottmann et al., 2020). Egg quality can be affected by the amount of lipids accumulated in the egg during its formation (Bobe and Labbé, 2010). High quality unfertilized eggs from SPE females contained a higher amount of total lipids, however this difference was not observed in the low quality group. Interestingly, fertilized embryos from females treated with a constant dose of CPE had a higher amount of lipids than SPE ones (Benini et al., 2022). This suggests that not only the hormone source but also the administration scheme can affect the amount of nutrients that are deposited in the oocytes during vitellogenesis. The levels of some saturated and monounsaturated fatty acids differed between hormonal treatments within the high egg quality group. Previous studies found no influence of SPE vs CPE on SFA and MUFA in European eel embryos and larvae, while MUFA levels were shown to decrease during embryonic and larval development (Benini et al., 2022). In this regard, the initial differences found in this contribution between treatments might not persist in later stages.

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GLOBAL WARMING CHALLENGES FOR AQUACULTURE: THE CASE OF INDIAN MAJOR CARP ROHU, *Labeo rohita*

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Introduction

Among the environmental factors, temperature plays an important role in the molecular, biochemical and physiological properties of animals (Pankhurst and King 2010; Strussmann et al. 2010). The survival, distribution, metabolism and reproduction of fish are all contingent on water temperature (Shahjahan et al. 2013; Shahjahan et al. 2017; Rahman et al. 2019). Increase of water temperature as a consequence of global warming is anticipated to affect the physiological activities of fish, especially in tropical regions. To understand the effect of high temperature on embryogenesis and growth, the commercially important rohu carp (*Labeo rohita*) was selected for the present study.

Materials and Methods

In the first trial, embryos and larvae were reared at four different temperatures (30, 32, 34 and 36°C), and indices of their hatching, development, and mortality were observed throughout early development. In the second trial, growth performance of fingerlings was evaluated after rearing in the three temperature conditions (30, 33 and 36°C) for 60 days.

Results

Embryos exposed to 30 and 32°C showed normal development with highest rates of hatching success. Embryos at 34°C displayed evidence of damaged zygotes, cellular deformities, damaged yolk sac coupled with shortest incubation time and the lowest rates of hatching success. No hatching was observed at 36°C. Larvae of rohu exposed to 34 and 36°C showed developmental deformities (fusion in the eye, axial curvature, yolk sac ulceration, blood coagulation, tail shortening and ulceration) and minimal survival. The growth parameters - final weight gain, percent weight gain and specific growth rate were the highest at 33°C and the lowest at 36°C. Lowest FCR value was found at 33°C. The effects of high temperature on hemato-biochemical parameters and erythrocytes cellular and nuclear structures in rohu were studied. Exposure to high temperature decreased Hb and RBCs and increased the WBC and blood glucose levels of the fish. Frequencies of ECA and ENA were found to be increased at high temperature. High temperature significantly increased the number of neutrophils whilst decreased the number of lymphocytes.

Conclusion

Overall, this study confirmed that exposure to high temperature is stressful to Indian major carp rohu.

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TESTING HYDRODYNAMICS EFFECT ON FLOW WITH CFD MODELING TOOL: CHANGING DIFFERENT INLET NOZZLES AND WATER DIVIDERS ANGLE IN A SEMI-CLOSED CONTAINMENT FLOATING TANK WITH RIGID WALLS

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Introduction

One third of the global salmon production is currently contributing by Norway. To improve the focus on the environmental performance of fish farms in Norwegian aquaculture, new businesses and innovative technologies are frequently introduced and tested across the whole country [1, 2]. One of the innovative production systems is Semi Closed-Containment Systems (S-CCS), where the fish are separated from the outside natural environment with a physical barrier. With a proper enhanced control on tank hydrodynamics and self-cleaning, production cycle, disease transmission and environmental impact which makes S-CCS a favorable replacement to open-cage production systems [1, 3]. The overall hydrodynamic performance of the system is influenced by inflow characteristics, i.e. turbulence produced by inlet orientations but experimentally it is not feasible to study velocity, uniformity, vorticity and swirl number, respectively [5,6]. Therefore, computational fluid dynamics modeling (CFD) is considered as a most appropriate tool to investigate the hydrodynamics of such a large system.

The aim of current CFD investigation was to monitor the effect of inlet column nozzles orientation angle and water dividers placements on the hydrodynamics of S-CCS with rigid walls and establish the optimal inlet column nozzles orientation set-up for use during Atlantic salmon production in this system.

Materials and Methods

Circular shaped floating tank with rigid walls and a total volume of 3500 m^3 received water from two inlet column with nozzles (possible to operate 6 inlets columns simultaneously). The water is discharged through central outlets located at a depth from top **(between -1.5m to -5.5m)** (sieved region of 4m around the central outlet pipe). In order to design and find the most optimal inlet nozzles and water divider (V-shaped closed structure placed behind each column to split water stream) orientation angle for optimal water distribution, a set-up of four cases were developed in CFD (Table 1). The hydrodynamics of the system was evaluated using different flow field indicators, such as flow velocity, distribution of vortices, turbulence in the system and vorticity.

Naiver Stokes equation for incompressible fluids was solved with SIMPLE algorithm, which is Semi Implicit Method for Pressure Linked Equations._Where initially pressure and velocity values are estimated by algorithm and later pressure-correction equation $\nabla^2 p\nabla=1/\Delta t\ (\nabla\cdot V)$, is solved to obtain a corrected value of pressure and velocity field and at the solution convergence is checked. A k-omega SST turbulence model with first order accuracy in space and time is used to solve Turbulence Kinetic Energy (k) and Specific Dissipation Rate (ω) [4]. In present study, one assumption in selection of boundary conditions is that no external force factors are included in the system (sea waves effect).

Results

In all 4 CFD simulations cases are compared at different depths (-1, -3, -6, -9), respectively. Velocity and mixing pattern show hydrodynamic difference in flow field across the tank. Out of 4 analyzed cases, the best hydrodynamics in the system (Figure 1a and 1b) was achieved by case 1 set-up (Table I), while the large variation in hydrodynamics was observed between cases. Observed differences in velocities between CFD models and empirical measurements were further investigated.

Discussion and conclusion

To investigate the hydrodynamics of large systems, we compared different design and Computational models, respectively. The understanding of two inlets flow patterns plus extra water dividers installments behind all 6 pipe columns could be performed more efficiently and relatively cheaper with the help of CFD modeling and simulations. In order to achieve a good qualitive and more reliable results, it is very important to develop a Solid CFD bench model. In this study, initially we developed a reliable CFD bench model and then examined the complete flow patterns for 4 cases with different inlet nozzles and water dividers orientation angles (Table I). Large differences between the flow patterns are observed among various cases, based on their inlet orientation angle change. This factor has large impact on mixing and velocity factor across the system, which in turn effect water quality for optimal fish growth/welfare, health performance and particle removal. This study shows that further optimization of the system set-up is achievable with the help of CFD modeling.

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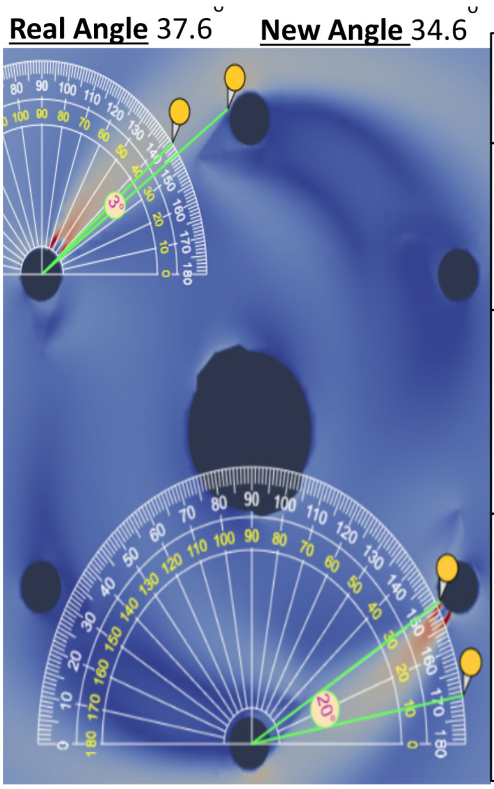
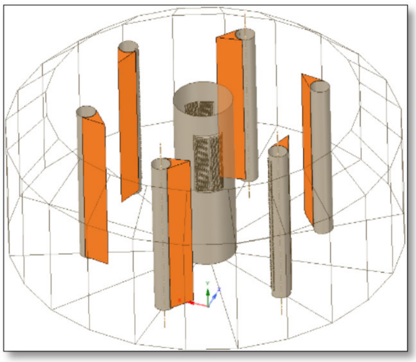


1st Case Real Angle 37.6°
2nd Case Water divider Angle 30°
3rd Case New Water Divider Angle 10°
4th Case (Water divider at Angle 10° + 3° change in nozzles angle)

Table (I). Different case setup configurations

Cases Setup	
1	Original Design
2	Water divider at Angle 30°
3	Water divider at Angle 10°
4	Water divider at Angle 10° + 3° change in nozzles angle



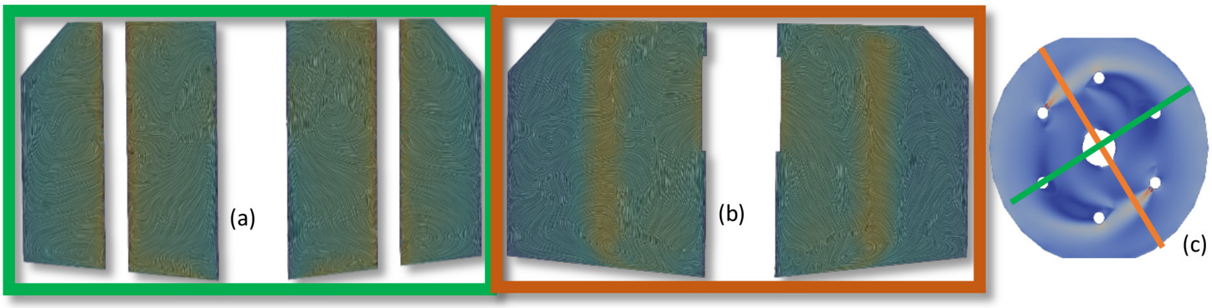


Figure 1 (a,b) CFD velocity mapping slices at different orientation (slice position shown by green and orange color line in image (c)) Top view of tank show two intake pipes orientation based on case 1 is shown.(c) slice is represented by green and orange line, which is a cut on certain position and shown as surface in (a, b)*

Acknowledgements
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DISSOLVED NUTRIENT EXCRETION IN NILE TILAPIA (*Oreochromis niloticus*) AND AFRICAN CATFISH (*Clarias gariepinus*): INVESTIGATING PROTEIN SOURCES IN FEEDS FOR CIRCULAR MULTITROPHIC FOOD PRODUCTION SYSTEMS

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Introduction

With the goal of transcending recirculating aquaculture systems (RAS) and aquaponics, the CUBES Circle project [1] investigates the possibilities of combining the production of fish, plants and insect larvae to create a fully integrated land-based multitrophic food production system with the objective of increasingly closing internal nutrient cycles and thereby minimizing waste. This means to use internally produced solid and dissolved nutrients as effectively as possible by continuously recycling them through the three trophic levels, i.e. dissolved nutrients from fish production support plant growth, solid wastes from fish and plant production build the base for insect larvae nutrition and insect larvae as well as slaughter waste from fish production reenter the production cycle as ingredients of the fish diet. Fish diets for such a system need to not only ensure optimal fish performance and health but also have to be optimized for the provision of nutrients downstream to plants and insect larvae. Therefore, the first basic question posed was how different protein sources of the circular bioeconomy as alternative to fish meal (black soldier fly larvae meal, poultry by-product meal, poultry blood meal) affect growth as well as dissolved nutrient excretion (N, P, K) of the CUBES Circle candidate species Nile tilapia (*Oreochromis niloticus*) [2] and African catfish (*Clarias gariepinus*) reared in RAS (i). Based on these results, the following question was then how specific diets could be formulated for different plausible production scenarios to most effectively close internal nutrient cycles. Accordingly, internally available protein sources (insect larvae meal, slaughter by-product meal) were combined with additionally required external protein sources to formulate specific experimental diets that were compared to an industrial standard diet to judge their suitability to ensure adequate fish production while improving dissolved nutrient profiles for plant production (ii).

Material & methods

i) Four isolipidic and isonitrogenous (40 % crude protein, 12 % crude fat) single protein source diets were formulated with fish meal (FM), black soldier fly larvae meal (BSFM), poultry by-product meal (PM) and poultry blood meal (PBM) as the main protein ingredient, respectively, and fed to Nile tilapia and African catfish (initial weight 10 g) reared in RAS in two separate 7-week trials (n=4).

ii) Three isolipidic and isonitrogenous experimental diets were formulated on the basis of an industrial standard feed for African catfish (46 % crude protein, 19 % crude fat). All of the experimental diets included 13.3 % catfish by-product meal (CM) and 10 % PBM as protein sources supplemented by 1) PM, 2) BSFM and c) a 50:50 combination of PM and BSFM. Experimental diets and the industrial diet were fed to African catfish reared in RAS in a 10-week trial (n=4).

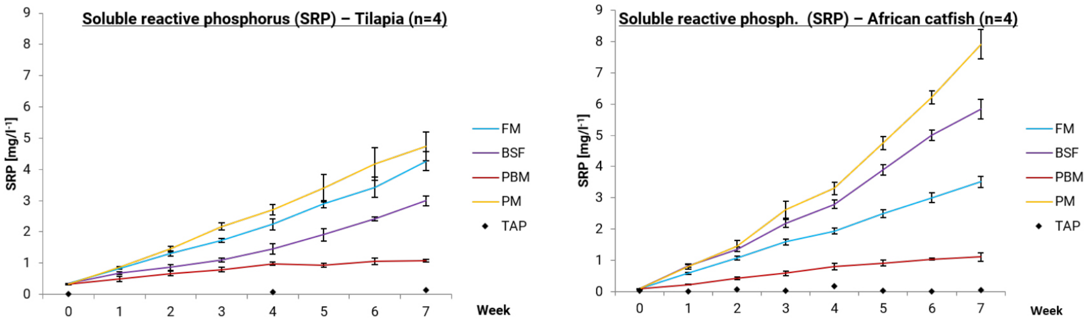

Figure 1. Accumulation of SRP in the RAS water over the period of the respective trials involving Nile tilapia (left) and African catfish (right).

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For all of the experiments above, dissolved nutrients in the process water were analyzed weekly (continuous flow analysis (CFA), inductively coupled plasma optical emission spectrometry (ICP-OES)) and growth performance was recorded at the end of the trial.

Results

Results of the first trials (i) showed that especially PM as the main protein source in diets for Nile tilapia and African catfish can match (in case of the tilapia) or at least come close to (in case of the catfish) a diet entirely based on fish meal in terms of growth performance and feed conversion. However, fish fed the BSF and PBM diet performed significantly worse regarding these measures. With respect to soluble reactive phosphorus (SRP) in the RAS process water, the PM feed resulted in the highest accumulation over the period of the trials for both fish species, with the differences being significant for African catfish. The accumulation of K was substantially and significantly higher for the BSF diet for both species.

Results for the follow-up trial (ii) are currently being generated and will be added to the oral presentation.

Discussion

The results support the general consensus that PM is a suitable protein source in Nile tilapia and African catfish nutrition and diets with PM can, even at high inclusion levels as the sole main protein source, rival the growth and feed conversion performance of an entirely fish meal-based diet. In addition, the PM diet produced higher dissolved SRP accumulation in the process water for both species which makes it an appropriate candidate for aquaponic diets which aim to increase phosphorus availability to plants. The BSFM, even though not enabling the same growth performance as PM and FM for both species, appears to be a rich source of dissolved K, also often insufficiently available in aquaponic waters. If combined with complementary protein sources to improve fish performance and still incorporated at sufficiently high levels, BSF meal could present an important ingredient in diets for aquaponics and multitrophic systems. As alluded to, the strategic supplementation of important protein sources which are internally generated in circular multitrophic food production systems (CM, BSFM) with externally obtained protein sources (PM, PBM) will subsequently yield further valuable insights into the opportunities of beneficially manipulating dissolved nutrient profiles.

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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED MIRNAS AND THEIR PREDICTED TARGET GENES IN GILL INDICATES THAT ATLANTIC SALMON SMOLTIFICATION AND SEAWATER ADAPTATION IS POST-TRANSCRIPTIONALLY REGULATED BY MIRNAS

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Introduction

Smoltification is a complex developmental process that leads to changes in the fish physiology, morphology and behaviour including remodelling of the gill from an ion-absorbing epithelium to an ion-secreting epithelium [1-3]. Optimal smoltification is, therefore, crucial for normal development, growth, and health of farmed Atlantic salmon in seawater phase [4]. The aim of this study was to explore whether miRNAs are involved in regulation of gene expression during this important developmental transition by characterizing miRNAs differentially expressed during smoltification and the following seawater adaptation (SWA) in gill of Atlantic salmon.

Material and methods

Total RNA from Atlantic salmon gill collected before smoltification (T1), halfway through smoltification (T2), three quarters into smoltification (T3), one day prior to seawater transfer (SWT) (T4), one week after SWT (T5) and one month after SWT (T6) were used to study expression changes of miRNAs and mRNAs. Small-RNA sequencing and microarray analysis were applied to identify miRNAs and mRNAs that showed significant differences in expression when groups from the different time points were compared. In silico prediction of miRNA-target-genes were carried out applying the MicroSalmon GitHub repository (http://github.com/AndreassenLab/MicroSalmon/) [5] with differentially expressed miRNAs (DE-miRNAs) and differentially expressed mRNAs (DE-mRNAs) as input. Finally, gene ontology and pathway enrichment analysis of predicted DE-miRNA targets were conducted using PANTHER Overrepresentation Test (http://www.pantherdb.org/) to reveal significantly enriched biological processes and gene pathways associated with smoltification and SWA.

Results

In total, 32 mature miRNAs were differentially expressed, and 18 of these mature miRNAs were characterized as the biologically important guide miRNAs (gDE-miRNAs) by examining the relative abundance of the 5p and 3p miRNAs originating from same precursors [6]. Hierarchical clustering analysis of gDE-miRNAs showed two major clusters, one with a decreasing and one with an increasing expression during smoltification and seawater adaptation. The gDE-miRNAs were predicted to target 747 of the genes that showed significant differences in their expression in the microarray analysis. Enrichment analysis of these predicted target genes revealed that they were enriched in several biological processes including processes associated with immune system (e.g., regulation of macrophage derived foam cell differentiation, regulation of dendritic cell antigen processing and presentation, viral entry into host cell and T cell activation), regulation of cell growth and lipid metabolic process (e.g., tissue regeneration and steroid biosynthetic process), reactive oxygen species metabolic process and response to stress. Additional pathway enrichment also showed that pathways associated with immune system (e.g., cytokine signaling in Immune system, neutrophil degranulation and adaptive immune system), extracellular matrix organization, signal transduction and metabolism of lipids, vitamins and cofactors as enriched.

(Continued on next page)

Discussion and conclusion

The finding that some enriched biological processes and pathways were associated with immune system, were as expected as gills are the main mucosal surfaces and immune barriers where several immune related cells including the coordinated expression of cytokines are presented [7]. The gill mucus cell population has also been reported to increase in number in response to increased salinity [8]. Regulation of cell growth and steroid synthesis may play a role in smoltification-associated osmoregulatory changes as growth hormone and cortisol have been suggested to stimulate salinity tolerance and NKA activity by promoting the formation of the seawater mitochondria-rich cells or chloride cells [9,10]. Gene ontology group response to stress was also enriched, which is in agreement with previous study that reported that stress response genes were upregulated in the gill of Atlantic salmon in response to smoltification and SWA [11]. The findings that all transcripts enriched in these biological processes were predicted targets of the gDE-miRNAs, indicate that miRNAs are involved in post-transcriptional regulation of gene expression changes that are crucial for remodelling of the gill during this developmental transition. Furthermore, this study points out pairs of gDE-miRNAs and their predicted targets that may be validated and further studied by functional assays to explore their particular role in the smoltification process.

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CASE STUDY OF GEOSMIN CONTROL IN FRESHWATER RECIRCULATING AQUACULTURE SYSTEMS FOR ATLANTIC SALMON

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Introduction

The total food fish consumption has increased globally over the past decades. Over the same period, aquaculture has become the main aquatic food producer as capture fishery production is decreasing due to overfishing and water pollution. Aquaculture provides food for a growing population and is a major source of essential nutrients. Despite these advantages, traditional intensive outdoor aquaculture systems can have serious negative environmental effects as for example eutrophication, the spread of disease or the release of chemicals. Therefore, new aquaculture production technologies are needed. Land-based recirculating aquaculture (RAS) fish and seafood production is a possible alternative. Potential advantages of RAS fish production are, for example, controlled breeding conditions, optimized water quality and disease control or lower environmental impact. RAS as new technology has several limitations and disadvantages which require further research and development. These challenges include high capital and production costs, high energy consumption, the necessity for continuous performance monitoring, difficulties in treating diseases, or a need for a high level of technological and biological know-how due to the complexity of the system and its interactions. One major challenge in RAS production is the accumulation of off-flavour (taste and odour causing compounds) in fish filets. The main objective of the study was to reduce geosmin concentrations in RAS rearing water for Atlantic salmon *(Salmo salar)* production and, by this, also reduce the accumulation of geosmin into the fish fillet.

Methods

A commercial scale freshwater RAS for Atlantic salmon production was monitored over several months in high resolution with GC-MS for geosmin in water before and after the installation of a protein skimmer (with ozone) in combination with a UVC system. Water parameters of the RAS and flow through purge system were monitored over the whole measurement period.

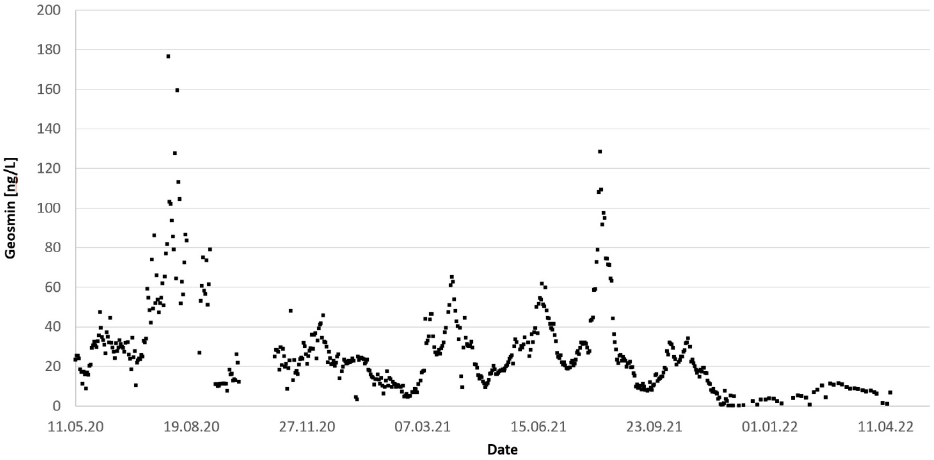

Figure 1: Geosmin concentration in Atlantic salmon freshwater RAS grow out process water over a measurement series of 23 months.

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Results

Before the use of ozone treatment, together with protein skimming and UVC, geosmin concentrations fluctuated in a daily resolution between around 5 ng/l and 180 ng/l. A by-monthly fluctuation is visible, which correlates with biomass transfers within the farm. After the start-up of the additional treatment loop and a last peak of geosmin, the average geosmin concentration dropped but still fluctuated and stabilized at a low level between <1 ng/l and 11 ng/l (see Figure 1). These low levels of geosmin in process water results in a low depot of geosmin for fish to be purged, reducing the needed purge time, weight loss and water consumption.

Conclusion

Technical approaches to control the growth of geosmin-producing microorganisms and improve the removal of geosmin can reduce geosmin concentration in grow-out freshwater RAS with a high effect.

RED KING CRAB (*Paralithodes camtchaticus*) IN AQUACULTURE: OPPORTUNITY AND CHALLENGES

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Introduction

Red king crab (RKC, *Paralithodes camtschaticus*) has become important for the shellfish industries located in the northern parts of Norway. In fact, in 2021, 2261 metric tons of RKC was exported from Norway, amounting to 999 million (NOK), respectively. About 60% of the exported volume was traded live whereas the remaining share was exported as processed. Catches of king crabs have declined globally due to overfishing, which led to an increased focus on RCK farming in Norway. When you start with new species in aquaculture, it is important to determine the optimum environmental conditions such as temperature, water requirement and stocking density. Furthermore, it is important to develop new feed and technological solutions for live holding of RCKs for economic and animal welfare reasons.

The present study was undertaken to study how different enviromental conditions effect the growth and survival of the animal.

Material and methods

After capture in northern Norway, the RKC was transported to the Research Station in Tromsø, landed, and kept in tanks. To screen potential effect of different environmental conditions, parameters like water requirement, temperature, stocking density, feed, duration, and the time of the year, varied. During the period for aquaculture, the RKC was sampled and evaluated. The evaluation included growth, survival, water quality, metabolism study and evaluation of behavior of the animal.

Conclusion

In general, RCK have low water requirements, low feed intake, tolerate high individual densities and shows low negative social behavior compared to lobsters that must be kept individually. In case of farming RKC, we have demonstrated that increasing temperature and stocking densities increases the risk of cannibalism, mortality, and injuries. Furthermore, it is important to develop new technological solutions for live holding of RCKs on and improve the dry feed (texture and nutrition) for improving FCR and the animal welfare reasons.

MICROPLASTICS CONTAMINATION IN A VARIETY OF CANNED SEAFOOD WITH APPLICATION OF AN OPTIMIZED METHODOLOGY

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The global production of plastics has continually increased from 1.5 million tons in 1950 to approximately 370 million tons in 2020, and a worldwide interest is growing on microplastics (MPs: plastic particles smaller than 5 mm) occurrence and impacts in marine environments. Seafood has a crucial importance for human consumers because it is an important source of high-quality proteins (including all the essential amino-acids), unsaturated fatty-acids (including Omega-3), fat-soluble vitamins and minerals, with numerous nutritional and health benefits. Thus, understanding the potential contamination of seafood with MPs is critical for food security and human health. Seafood is also highly perishable, and several preservation methods (like salting, freezing, or canning) are traditionally used to ensure the quality of the products. Canned seafood corresponds to approximately 10% of the total 178 million tons of the world fishery production, with the existence of a wide variety of canned organisms (*e.g.,* fish, molluscs, crustaceans), immersed in different edible liquids (*e.g.*, sunflower oil, olive oil, tomato sauce), each one with specific ingredients and nutritional facts (*e.g.*, fat, carbohydrates, proteins). Since consumers eat these products without any additional cleaning process, from a health perspective it is crucial to have a better understanding on possible MPs contamination both in the food tissues, as well as in the respective immersive liquids. The occurrence of MPs has been widely studied for several seafood species but, regarding canned seafood, there is still limited information. Thus, in the present work, a variety of canned organisms (n= 60) immersed in different types of oils and sauces were studied: tuna in olive oil, sardine in sunflower oil, sardine in tomato sauce, chub mackerel in sunflower oil, octopus in tomato sauce, and mussels in escabeche sauce. Samples were digested with 30% H_2O_2, followed by vacuum-filtration, and subsequent observation under a stereomicroscope. Recovered MPs were photographed and stored for posterior polymers analysis in FTIR (Fourier-transform infrared spectroscopy). The results showed that 45% of the samples had at least one microplastic, with all the studied organisms presenting MPs contamination. A total of 40 MPs items were found (Fibers= 30, Fragments= 7, Film= 3), with an average of 0.04 MPs/g of seafood tissue (n= 26), and an average of 0.01 MPs/g of seafood liquid (n= 14). The most contaminated seafood was the canned tuna, with an average of 0.11 ± 0.10 MPs/g, while canned sardine in sunflower oil was the less contaminated, with an average of 0.02 ± 0.04 MPs/g. FTIR analysis is still undergoing, and polymer cancerization of MPs observed will be used to try to identify contamination sources. Our results demonstrate that canned seafood is contaminated with MPs and constitutes a potential contamination pathway for humans, highlighting the importance of quantifying and regulating MPs in canned seafood, to increase food safety and human health.

Acknowledgements

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MONITORING MICROBIAL DYNAMICS IN A PERCH RECIRCULATING AQUACULTURE SYSTEM WITH FULLY AUTOMATED ONLINE FLOW CYTOMETRY

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Introduction

Microorganisms are omnipresent and crucial players in all aquatic systems. They can have a large impact especially in recirculating aquaculture systems (RAS), where water is reused continuously. In these systems, tight control on water quality is essential for successful and safe fish production. Microorganisms affect fish health in a multitude of ways and control of a RAS is inextricably linked to microbial concentrations, be it in the fish tanks where pathogen growth must be avoided, or in the biofilter, where microbial populations must be maintained abundantly for nitrification.

Online sensors are standard in the industry for multiple physicochemical parameters, however microbial concentrations are difficult to get by for fish farmers. Tracking of microbial dynamics requires sampling and analysis at very short intervals and ideally in real-time allowing for immediate interpretation/reaction. For decades this was impossible with conventional microbial cultivation-based methods but also with advanced molecular methods, which are still too labour-intensive, time-consuming, and costly for such applications.

One promising approach is the use of flow cytometry, which allows fast, accurate and reproducible quantification and differentiation of total and intact cells. This detection method has specific advantages including: rapidness, sensitivity, reproducibility, accuracy in quantification, and differentiation of total and intact cells.

Material and methods

In a first step, grab samples across the RAS were analysed by our flow cytometry protocol for total and intact bacteria. A perch and a carp cultivation were compared in terms of microbial concentration. In a second step, we used a fully automated online flow cytometry system that overcomes the tedious and restricting practice of grab-sampling and subsequent cultivation on agar plates Besmer et al. (2014). This system was used to monitor a perch RAS cultivation system. In short, water samples were drawn directly from several points in the system, mixed with a fluorescent stain, incubated, and then measured by flow cytometry. Rinsing and extended cleaning were performed regularly and periodically respectively. The resulting large sets of flow cytometry data were batch processed with custom software.

Results

Flow cytometry could successfully be used for the measurements of the grab samples. Background could be separated well, and the measurements were technically highly reproducible. The measurements showed significant concentrations of total and intact bacteria across each of the RAS, on the order of 10^5 to 10^6 cells/ml. Strikingly, while clear differences were detected between the carp and the perch cultivation systems, the concentrations across different locations within each of these systems were very similar.

The RAS is being monitored for an extended period, initiating one week prior to the fish entering the tanks, and continuing during the fish cultivation period. Changes in concentration of total and intact bacteria are being followed and compared with important process parameters like feeding and cleaning procedures.

Discussion and conclusion

Our results show the potential of advanced monitoring of microbial dynamics, which is critical for a better understanding of underlying causes of fluctuations as well as the ecological and operational consequences thereof.

We expect that these findings will massively enhance process monitoring, water treatment design and improvement (e.g., disinfection in RAS). In addition, we expect a potential for optimisation of feed and other production processes, including conceptual approaches to smarter sampling schemes, but also new applied and fundamental research directions.

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FISH LOGISTICS; THE KEY TO SUCCESFULL CLOSED CYCLE TUNA AQUACULTURE

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Introduction

Next Tuna GmbH is planning, the first of its kind, production facility for Atlantic Bluefin Tuna (*Thunnus thynnus*) (ABT) at the shore of the Mediterranean Sea. (see as well https://www.nexttuna.com/)

Tuna in general and specially ABT is very sensitive to handling. In the early live stages, the small fish are nearly impossible to touch, and at later stages, the fish are simply too big to be handled in a meaningful way.

Thus, internal fish logistics in the Next Tuna juvenile production concept becomes the main challenge to successfully produce ABT juveniles to a size of 2-10kg where they can be supplied into the existing Tuna fattening industry (see Fig.1).

In this contribution we want to present and discuss two novel approaches to internal fish logistics for tuna:

1. Internal transport in the hatchery by moving the entire tank around with a portal crane.
2. The use of floating RAS to facilitate transfer of the fish to transport net-pens, departing to Next Tuna customers.

Solution

<u>Hatchery internal transport by crane:</u>

For its hatchery operation, Next Tuna decided for movable tanks. The weaning and pre-growing tanks of Next tuna are planned in a way, that the whole tanks can be moved using a 40-tone overhead crane (Fig. 2). The bottom of the tank is equipped with an over-dimensioned bottom outlet to slowly release the fish. This avoids physical handling of the fish and adds a lot of flexibility in regards to fish density control. Another benefit of multiple movable units is a modular approach that will make it easy to refurbish and improve the systems for the specific needs of this species.

Floating RAS offer the advantage of land-based systems as long as they are connected to land, and in addition they offer the flexibility that they can be moved to the hatchery when loaded with juveniles, or joined with transport cages when fish are delivered to customers.

For fish delivery, the floating RAS is connected to the transport cages, comparable to the technology applied for fish transfer from one net-pen to another. Like this, the fish transfer from inside the RAS to the transport net-pen can be facilitate through a gate in the floating RAS side wall.

Discussion

Next Tuna wants to present these novel approaches to fish logistics to the audience and discuss the chosen solutions and their implications with the experts present at the conference.

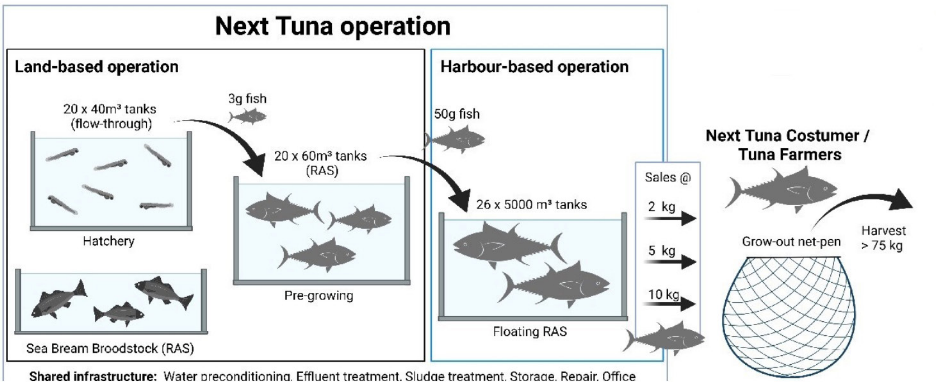

Fig. 1.: Schematic depiction of the Next tuna production concept.

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Fig. 2.: Example of a portal crane planned for the movement of fish together with the tank.

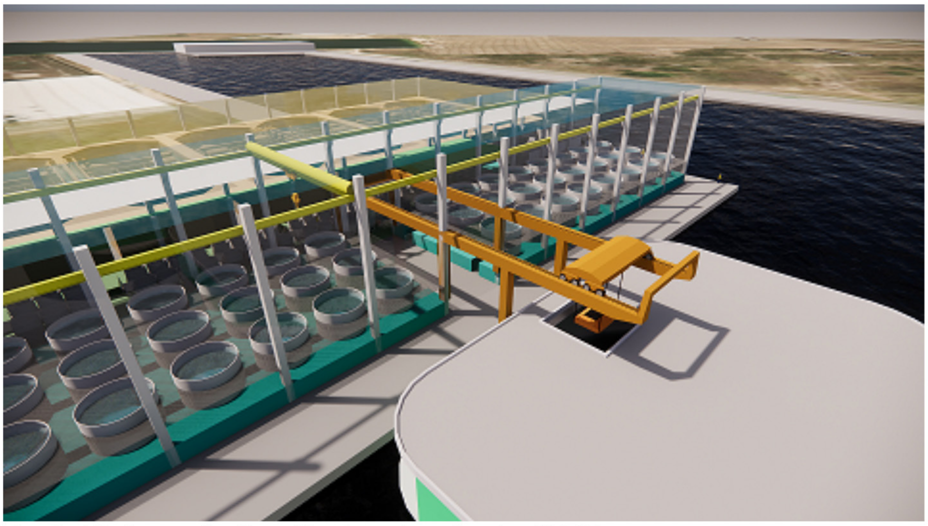

Fig. 3.: Fisch transfer from Hatchery into floating RAS by crane.

Ruditapes SP. AS BIOINDICATORS OF MERCURY PRESENCE IN PORTUGUESE COASTAL LAGOONS

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Mercury (Hg) is a pollutant that requires particular attention due to its high degree of toxicity, persistence and bioaccumulative properties. Although its anthropogenic emissions have been largely reduced in Portugal, historical contamination leads to considerable burdens of Hg in the sediments of Ria de Aveiro and Ria de Olhão. Bivalves molluscs, in particular, are widely used as biomonitors of environmental metal concentrations, due to their feeding behaviour, filtrating Hg from the water column, suspended and particulate organic matter. Besides, these organisms may act as vectors for the spread of Hg to higher trophic levels. Furthermore, bivalves' molluscs are traditionally used in gastronomy, and their harvesting represents an important activity in coastal communities. Therefore, monitoring Hg's presence along the Portuguese coast is extremely relevant to both environmental and food safety questions. The aim of this study was to assess Hg concentrations in bivalve molluscs collected monthly in aquaculture areas of three coastal lagoons (Ria de Olhão, Ria de Aveiro and Lagoa de Óbidos, April 2019 – January 2020). In this sense, samples of two species of clams (*Ruditapes decussat*us and/or *Ruditapes philippinarum*) were collected and, the Hg quantification was carried out through the DMA-80 Evo Direct Mercury Analysis System. Clams proved to be good bioindicators of Hg presence. As expected, clams from Olhão and Aveiro lagoons presented the higher levels of Hg than Óbidos lagoon throughout the year. However, values obtained for these clams are below the maximum levels stablished by European legislation of 0.5 mg/kg. Fluctuations on Hg accumulation due to seasonality will also be presented and discussed.

PREDICTING EPA AND DHA DEPOSITION IN TURBOT (*Scophthalmus maximus*) FILLET WITH A NUTRIENT-BASED AND FATTY ACID DILUTION MODEL

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Introduction

Concerns about sustainability have driven the evaluation and use of alternative lipid sources to replace fish oil (FO) in marine fish diets. However, total replacement of FO by other alternatives, such as vegetable oils (VO), is still hampered by the relatively low levels of long chain polyunsaturated fatty acids (LC-PUFAs; e.g., DHA and EPA) available in VOs. In addition to the fact that LC-PUFAs are required at considerably high levels by marine fish species to sustain adequate growth, they also make fish an attractive product for consumers, as it is considered a high-quality source of these essential fatty acids. In this sense, from the fish farmer point of view, delivering fish rich in LC-PUFAs, namely EPA and DHA, is a relevant concern, which may also contribute to the differentiation of their products in the market.

Mathematical models that support the definition of more efficient finishing feeding strategies, by predicting the deposition of EPA and DHA in fish flesh, may be useful tools for fish farmers, since the fatty acid (FA) composition of fish depends on several factors that may vary substantially during fish farming production.

This work aims to present the development and validation of a mathematical model, within the scope of the OmegaPeixe project, which was designed to support the evaluation of different feeding scenarios of turbot (*Scophthalmus maximus*) by measuring the impact on the EPA and DHA composition of the fish fillet.

Methodological approach

The mathematical model was developed based on a "energy-protein flux" approach (EP model; Nobre et al., 2019). Being a dynamic model (ordinary differential equations, ODEs), the whole-body content of protein, lipid and FA of fish are modeled as state variables. The FA deposition is modeled based on simple dilution model (Robin et al., 2003), assuming that the dietary FA incorporation occurs in tissues without the mobilization or turnover of pre-existing ones, i.e., in the same proportion as they are in the diet (Benedito-Palos et al., 2009). In order to convert the EPA and DHA composition from the whole-body to the fillet, the following equation was used:

$$FA_{fillet} = (lipids_{wholebody} \times a) \times (FA_{wholebody} \times b)/100$$

where:

FA_{fillet}	is the EPA or DHA composition of the fillet, in g/100g
$lipids_{wholebody}$	is the whole-body lipid content, in % of total weight
$FA_{wholebody}$	is the whole-body EPA or DHA content, in % of total lipids
a	is conversion factor of lipids (from whole-body to fillet)
b	is a conversion factor of EPA or DHA (from whole-body to fillet)

The model was calibrated for turbot using data from a wide range of growing conditions (experimental data collected from scientific publications and experimental/production data shared by FLATLANTIC). In addition, the model was validated with independent datasets, including one relative to a trial carried out in the context of the OmegaPeixe project (results available below).

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Model validation

The model validation results are shown in Figure 1. These results are related to an *in vivo* trial carried out with fish weighing initially 141.9 ± 6.5 g, which were fed over 75 days with 6 experimental diets formulated with different lipid sources.

Overall, the model predicts with very good accuracy both the growth and the EPA+DHA composition of the fish fillet over a 75-day period, presenting a mean absolute percentage error (MAPE) of 5.0% and 12.3%, respectively.

Final remarks

This work illustrates the development and validation of a nutritional mathematical model that can be used to support the definition of finishing feeding strategies, aiming the production of fish rich in EPA and DHA. The results obtained in a first validation step are promising, however further validation is required in order to gain more confidence in the model. This is currently in the pipeline of the OmegaPeixe project.

Acknowledgements

This work is part of the OmegaPeixe project (POCI-01-0247-FEDER-069748), supported by Operational Program for Competitiveness and Internationalization (COMPETE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

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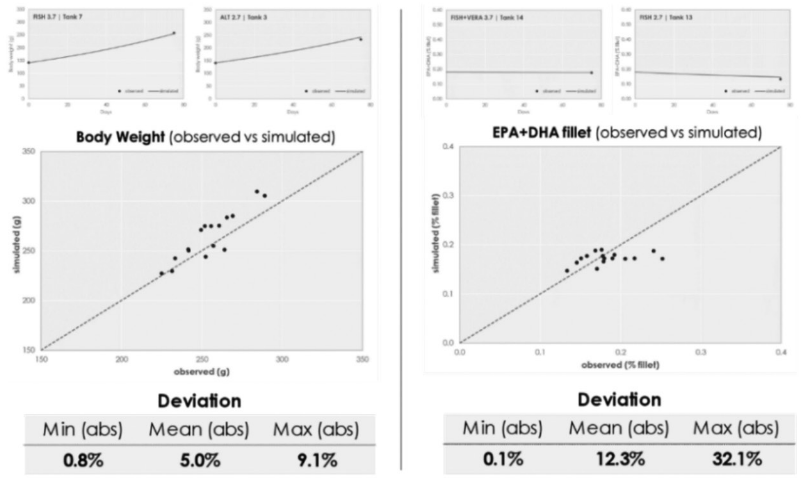

Deviation

Min (abs)	Mean (abs)	Max (abs)
0.8%	**5.0%**	**9.1%**

Deviation

Min (abs)	Mean (abs)	Max (abs)
0.1%	**12.3%**	**32.1%**

Figure 1 – Model validation for body weight and fillet EPA+DHA composition.

SOLEMAT: A MODELLING TOOL TO SUPPORT THE DEFINITION OF FEEDING STRATEGIES FOR SENEGALESE SOLE (*Solea senegalensis*) CULTIVATED IN RECIRCULATING SYSTEMS

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Introduction

Feeding operations have a substantial impact on the overall performance of recirculating aquaculture systems (RAS). The way fish are fed not only has direct consequences on the zootechnical and economic performance of fish production, but also has indirect consequences in terms of water quality. Under some conditions, these side effects, if neglected and not properly accounted for beforehand, can lead to undesirable negative consequences and, ultimately, pose a major threat to fish welfare and health.

Although most RAS are dimensioned to sustain a determined carrying capacity, it is important to bear in mind that the dimensioning is usually made by considering nutrient mass-balances that may not apply to all feeding strategies and farming conditions. For example, a fishmeal-free diet may lead to a 4-fold increase in phosphorus concentration, and to a 14% higher nitrate nitrogen concentration, compared to a fishmeal-based diet in a low water exchange RAS (Davidson et al., 2016). This illustrates the importance of evaluating the impact of new feeding strategies on the overall performance of the system, in order to ensure that there are no risks that could compromise production.

Decision-support tools based on mathematical models can be important assets in this regard. By predicting nutrient excretion over time and, hence, water quality parameters, based on a mass-balance approach, this kind of tools allow to gain more sensitivity and knowledge about the impact of different feeding scenarios on RAS performance.

The objective of this work is to present a prototype of a decision-support tool (SoleMAT), developed in the context of the FeedMi project (www.feedmi.ciimar.up.pt), which was designed to support the evaluation of different farming and feeding scenarios of Senegalese sole (*Solea senegalensis*) cultivated in RAS.

Methodological approach

The SoleMAT tool (Figure 1) was built on the top of a nutritional fish growth model, which is based on a "energy-protein flux" concept (EP model; Nobre et al., 2019), and which was calibrated for Senegalese sole using published data from several growth trials.

The EP model, scaled for the population/tank level, simulates what happens inside a tank, including the growth of fish, biomass evolution and total ammonia nitrogen (TAN) excretion, based on input data defined by the user about the feeding scenario to apply (i.e., feeds to provide over time, feed composition and feeding table) and expected farming conditions (e.g., temperature). For each tank defined by the user, the model is replicated and run in parallel. All EP model replicates are coupled to another model that simulates the removal/conversion of TAN into nitrites (NO_2) and nitrates (NO_3) by the water treatment system, thus allowing to predict the concentration of nitrogen compounds over time based on mass-balance approach that also takes into account the water renewal rate.

Tool overview

SoleMAT is implemented as a prototype tool through the integration of R scripts (compiled as executable files) and Microsoft Excel. It presents a user-friendly interface and requires a minimum set of input data (e.g., diet composition, feeding table, number of fish, initial fish weight, and temperature profile), thereby improving user-experience.

In terms of outputs, the tool provides not only time-dependent estimates of growth, biomass, FCR and nutrient excretion at the tank level, but also estimates on the water quality of the whole system, in terms of concentration of nitrogen compounds. Furthermore, this prototype tool allows the user to define critical concentration values of NH_3, NO_2 and NO_3, to facilitate the interpretation of results.

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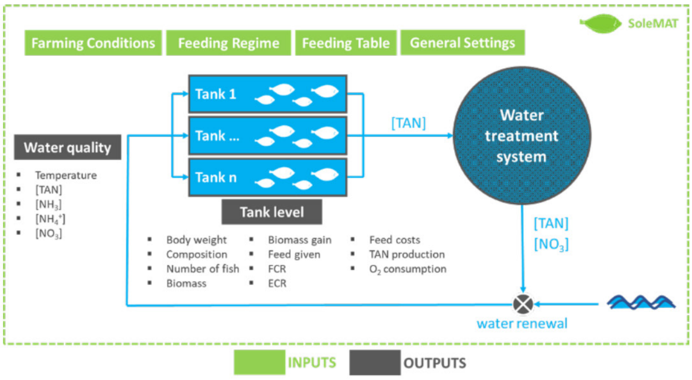
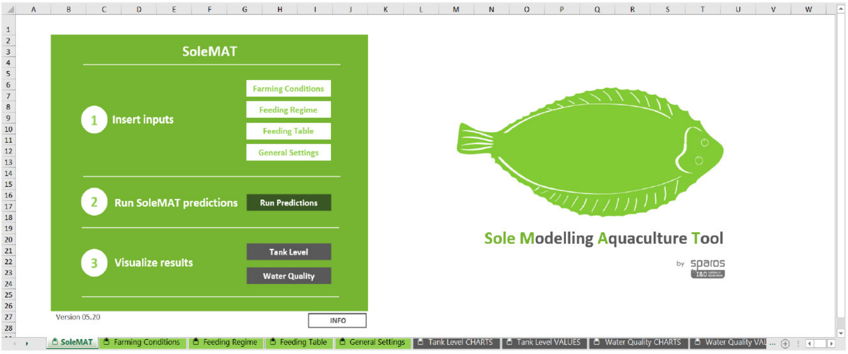


Figure 1 – Diagram detailing the overall concept of the SoleMAT tool.


SoleMAT

1 Insert inputs

2 Run SoleMAT predictions

3 Visualize results

Version 05.20

Figure 2 – SoleMAT main page, showing the typical user-flow.

Final remarks

This work illustrates how nutritional mathematical models can be used to build practical decision-support tools to support efficient feeding management practices in RAS.

Acknowledgements

This work is part of the FeedMi project (ALG-01-0247-FEDER-003520) supported by CRESC Algarve 2020, NORTE 2020, and the European Union through ERDF, in the framework of Portugal 2020.

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NOVIFEED – NOVEL TOOLS FOR INTELLIGENT FEEDING MANAGEMENT IN ATLANTIC SALMON (*Salmo salar*) AND MEAGRE (*Argyrosomus regius*) FARMING

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Introduction

Precision fish farming tools have a great potential to support the transition to more efficient fish farming production, particularly in the case of intensive systems. This kind of tools can be used to support different phases of the operational process, such as the observation phase, the interpretation phase, the decision phase, and the acting phase (Føre et al., 2018). Mathematical models can be inserted within the range of tools used to support the interpretation of data, allowing to act based on more informed decisions, thus contributing to improved performance in zootechnical and economic terms.

To contribute to an even more efficient fish farming industry, we have set up the NoviFEED project (www.sparos.pt/projects/novifeed), which aims to bring to market improved versions of the FEEDNETICS™ and FiT feeding tables™ tools for Atlantic salmon and meagre. Both tools are based on mathematical models that consider the effects of feed composition and temperature on feed intake, growth, nutrient retention and body composition of fish. With these tools users can evaluate the impact of different aquafeeds on their specific farming conditions and obtain the feed requirements for their targeted growth curve, allowing them to improve their feeding operations. Within the scope of NoviFEED, we will study the effects of dissolved oxygen on the voluntary feed intake of both species, aiming to include them on both tools through mathematical modelling.

This work aims to:

- Present the calibration and validation of the models behind FEEDNETICS™ and FiT feeding tables™ tools for Atlantic salmon (*Salmo salar*);
- Present other expected outcomes to be obtained in NoviFEED.

Model calibration and validation

The calibration of the models (ordinary differential equations, ODEs) behind FEEDNETICS™ and FiT feeding tables™ tools for Atlantic salmon was done by considering data from a wide range of growing conditions (experimental data collected from scientific publications, see Table 1) and by applying optimization algorithms that were used to define the values of some species-specific parameters based on an error-oriented approach (i.e., minimizing the error between observed and predicted data).

The validation of the models was performed by running them for independent datasets (i.e., out-of-sample data, not used in the calibration process) and comparing the results against observed data. This allowed a fair evaluation of model accuracy, avoiding some undesirable bias related to in-sample validation procedures. Figure 1 shows the validation results of FEEDNETIS™ (body weight comparison) and FiT feeding tables™ (feeding rate comparison) for Atlantic salmon.

Both tools provide reasonable estimates, presenting mean deviations of about 11.7% (FEEDNETIS™) and 14.0% (FiT feeding tables™) against the validation datasets tested. Despite these good results, through the project an additional set of *in vivo* nutritional trials will be carried out in order to generate detailed datasets, with the aim of refining the accuracy of both tools.

Expected outcomes of the project

The results presented in the previous section are the first outcomes of this project. Further work includes calibrating both tools for meagre (*Argyrosomus regius*) and studying the effects of dissolved oxygen on the feed intake of Atlantic salmon and meagre, through an extensive battery of short-term feeding trials (where feed intake will be recorded under different oxygen levels). If we are able to successfully observe clear effects of dissolved oxygen concentration on the feed intake, the next steps are to develop mathematical models to be included in new versions of both tools and demonstrate their use in operational environment to reach a complete and qualified system.

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Table 1 – Summary of calibration data used to calibrate the models for Atlantic salmon.

Number of rearing units and diets		BW and temperature range		Dietary composition range	
Number of rearing units	398	Body weight	1 – 6645 g	Crude Protein	29 – 54 %
Number of diets	350	Temperature	4 – 20 °C	Crude Fat	10 – 47 %

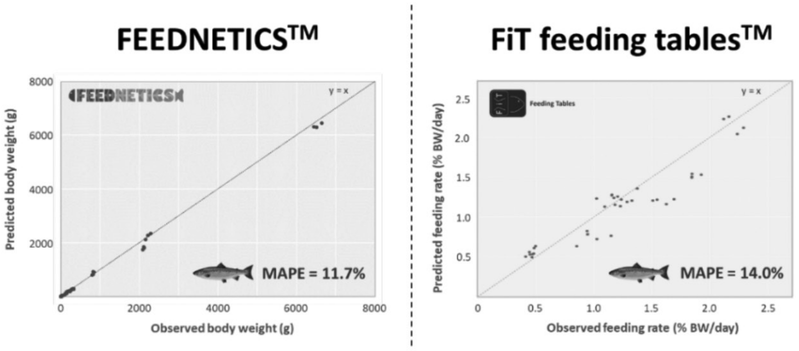

Figure 1 – Validation results of FEEDNETICS^{TM} and FiT feeding tables^{TM} for Atlantic salmon. MAPE = mean absolute percentage error

Final remarks

Nutrient-based mathematical models have great potential to contribute to precision fish farming, by providing, for instance, theoretical estimates of feeding rates and by allowing to compare different feeding regimes before field implementation. In order to extend the predictive capacity of this kind of tools, it is important to keep the development pipeline open to the inclusion of factors other than those related only to the nutrient content of feeds. Dissolved oxygen is a factor with a great impact on fish farming productivity. Therefore, tools that are able to quantify its impact on fish performance will allow to support the definition of more efficient feeding practices.

Acknowledgements

This work is part of the NoviFEED project, financed by Iceland, Liechtenstein and Norway, through EEA grants, in the scope of the program Blue Growth, operated by Directorate-General for Maritime Policy (DGPM), Portugal, under reference PT-INNOVATION-0099.

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DETERMINATION OF OYSTER SPECIES AND DISEASES FOR THE DIVERSIFICATION OF THE EMERGING SHELLFISH SECTOR ON THE BASQUE COAST (SE BAY OF BISCAY)

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Introduction

Offshore aquaculture is a promising economic activity in waters of the Basque Country (SE Bay of Biscay) as a complement of the declining fishing activity in the area. Since 2011 efforts have been focused on the feasibility of mussel (*Mytilus galloprovincialis*) aquaculture in open waters, but there is also interest in diversifying the production by cultivating new species. In this respect, oysters are particularly attractive since they present a high market demand. However, the oyster precise species inhabiting the Basque coast as well as the pathogen prevalence is unknown, which is of vital importance for oyster farming profitability. In fact, the prevention and control of diseases is one of the challenges that has to face bivalve mollusc production. Thus, the aims of this study are (i) to identify the species of wild oysters inhabiting the Basque coast and (ii) to determine the prevalence of oyster diseases.

Material and methods

The study was carried out in 2020 in six locations distributed along the Basque. At each location 30 wild oysters were collected to characterise the oyster species and other 30 individuals were collected to determine the prevalence of pathogens, except in Mendexa, located in the Mollusc Production Area, where pathogens were detected on 30 commercial flat oysters. Characterisation of oyster species was performed by PCR-sequencing of a specific DNA fragment, belonging to the mitochondrial cytochrome oxidase I (COI) gene using the primers LCO1490/HCO2198 (Folmer et al., 1994). Sequences were aligned using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with a >98% at reference species level. Pathogens were determined by qPCR (Herpesvirus-OsHV-1 μVar- and *Vibrio aestuarianus*) and PCR (*Mikrocytos mackini*) in the National Reference Laboratory for Diseases of Bivalve Molluscs, CSIC (Vigo, Spain), according to the methodology standardised by the European Reference Laboratory and/or by the Office International des Epizooties (OIE), renamed as World Organisation for Animal Health , and Bonamiosis and Marteiliosis diseases were determined in histological sections of commercial flat oysters.

Results

All wild oysters were identified as belonging to the genus *Magallana*. However, after sequencing the amplified fragments, one sample from the port of Mutriku was identified as *Magallana angulata*, while the rest of the samples turned out to be *M. gigas* (Figure 1). The European flat oyster (*Ostrea edulis*), the native species of European waters, was not found, except in the commercial batch reared in Mendexa.

Prevalence of herpesvirus ranged from 0% in oysters from Muskiz to 20% in oysters from Hondarribia. Accordingly, the highest prevalence values of *Vibrio aestuarianus* were recorded in oysters from Hondarribia (20%). *Mikrocytos mackini* was not found in any Pacific oyster from the Basque coast. On the other hand, a prevalence value of Bonamiosis of 3.3% was detected in commercial flat oysters from Mendexa but no Marteiliosis was registered (Table 1).

Discussion and conclusion

The vast majority of oysters from the Basque coast (99.3%) were identified as Pacific oyster (*M. gigas*), while no wild European flat oysters were found in the sampling locations. Oysters sampled on the Basque coast were infected to varying degrees by pathogens. However, it should be noted that the prevalence of the pathogens did not exceed 20% of cases, so for the moment they do not pose a major threat, but it must be taken into account that there is hardly any oyster farming activity in the Mollusc Production Area and that the density of farmed animals is low. If production increases in the future and more companies are set up, the risk may increase. It is therefore essential to develop a health surveillance plan to better prevent and control marine bivalve diseases and to promote a sustainable aquaculture.

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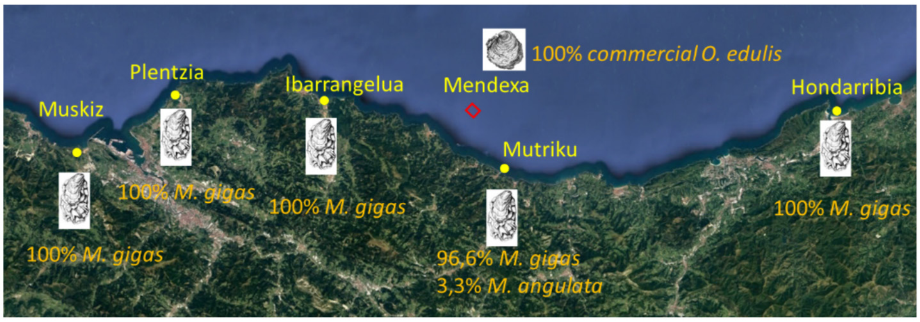

Fig. 1. Percentage of oyster species identified in the Basque coast.

Table 1. Prevalence (%) of pathogens identified in Pacific oysters of the Basque coast except in Mendexa where pathogens were determined in European flat oysters.

Location	**Muskiz**	**Plentzia**	**Ibarrangelua**	**Mutriku**	**Mendexa**	**Hondarribia**
Oyster origin	Wild	Wild	Wild	Wild	Commercial	Wild
Herpesvirus-OsHV-1 µVar	0	6.7	3.3	10	-	20
Vibrio aestuarianus	6.7	0	6.7	0	-	20
Mikrocytos mackini	0	0	0	0	-	0
Bonamia sp.	-	-	-	-	3.3	
Marteilia sp.	-	-	-	-	0	

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SUPPORT FOR THE MANAGEMENT OF THE EMERGING SHELLFISH SECTOR ON THE BASQUE COAST (SE BAY OF BISCAY)

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Introduction

Offshore aquaculture has been recognized as a potential solution to the increasing demand for local seafood, while maintaining marine ecosystems. In recent years, due to the decline of fishing activities in the Basque Country (SE Bay of Biscay), a study was conducted to validate the feasibility of farming mussels (*Mytilus galloprovincialis*) in the Cantabrian open waters. Although this study concluded on the viability of this activity, the operational production phase started in 2019 by the first mussel production company installed, is encountering some difficulties that compromises the economic profit of the activity. These difficulties are related to an increase occurrence of different harmful algae blooms and to the deficient follow up of the mussels' reproductive cycle. The present study addresses these issues in detail, to provide better knowledge and management support on the periods of highest mussel meat quality and seed provisioning, as well as identifying toxic events that may impede mussel marketing.

Material and methods

This study was conducted from January to December 2021, on the offshore long-line installations located at two miles off the Basque coast, presently exploited by a mussel production company. Samplings were carried out monthly for the determination of mussel growth and reproductive cycle based on histological assessment, together with the measurement of environmental variables. Additionally, the analysis of mussel flesh for the presence of the regulated biotoxins (Diarrheic, Paralytic and Amnesic Shellfish Poisoning toxins, together with yessotoxins and azaspiracids) and the analysis of water samples for the presence of toxin-producing phytoplankton throughout the water column was carried out at least fortnightly.

Results

Gametogenesis took place in early winter, using reserves stored during the previous months. Mussels remained mature during winter and spring and mass spawning occurred in late summer. Subsequently a post spawning period was observed from late summer to autumn. However, it is worth noting that a continuous spawning was observed throughout the year. In some mussels, gonad restoration occurred in autumn to reabsorb their gonads concurrently with development of storage tissue. The onset of a new gametogenic cycle was expected by late summer (Figure 1).

Okadaic acid, a "Diarrheic Shellfish Poisoning" (DSP) toxin was produced by *Dinophysi*s *acuminata* and exceeded the legal limit in spring, from the beginning of March to the end of June as observed in other areas (Ruiz-Villareal et al., 2016) (Figure 2). On the other hand, lipophilic toxins of the yessotoxin group exceeded the legal limit during several days in June and October. The rest of the regulated lipophilic toxins (dinophysistoxins, pectenotoxins or azaspiracids) were not detected. The amnesic toxin (ASP) and paralytic toxins (PSP) did not exceed the legal limit for domoic acid and saxitoxin, respectively.

Discussion and conclusion

Although the main spawning peak occurred in late summer, a noticeable proportion of gamete releasing mussels was still detected throughout the year, which could lead to a decrease in meat quality. Therefore, to increase profitability of the production, the summer months should be avoided for the sale of the product. Instead, producers should be encouraged to place ropes on the longline for seed collection. However, previous studies have indicated that spring and autumn are the seasons with the highest risk of biotoxin events (DSP and PSP, respectively), and, consequently, closure of the mollusk production area. As there are no accredited laboratories for biotoxin analysis in the Basque Country, more efforts are needed to consolidate the value chain of aquaculture products. In addition, the use of screening tests for early detection of biotoxins could help to identify product sales windows and improve the management of the emerging shellfish sector in the Basque Country.

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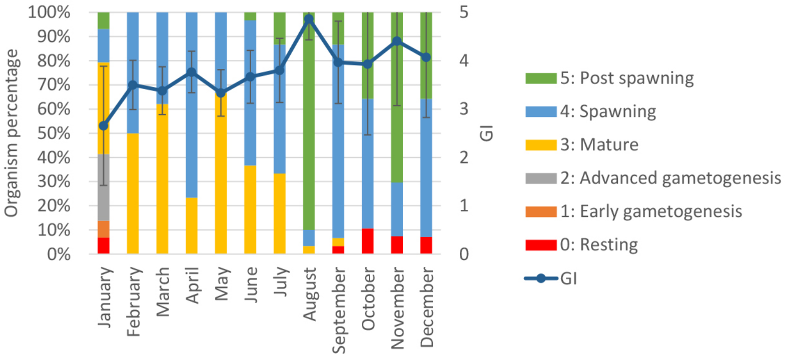

Fig. 1. The percentage distribution of gamete development phases (stacked bars) and gonad index (GI) values (blue solid line) in mussels from the Basque coast.

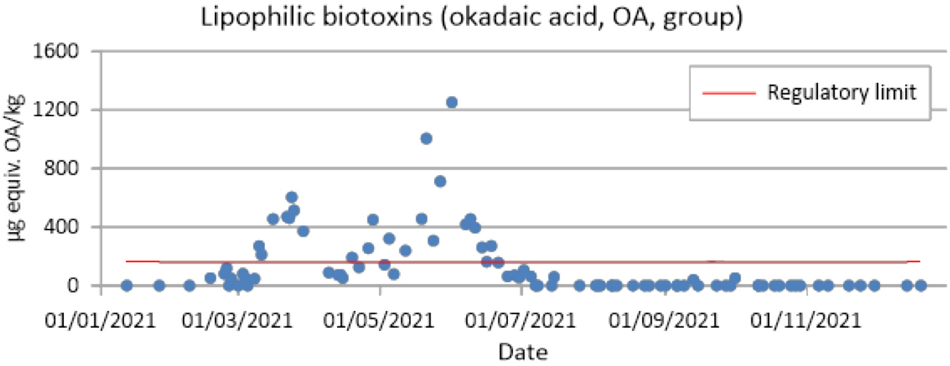

Fig. 2. Concentrations of okadaic acid (OA) in mussels

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AQUACULTURE TECHNOLOGY AND GOVERNANCE – GOING DOWN RABBIT HOLES?

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Introduction

Norwegian aquaculture has seen immense development. In less than half a century, it has developed from a fumbling, experimental activity; to a technologically refined, and highly efficient industry. In the shadows of its success, however, lie environmental impacts such as pollution, escapes, and sea lice. In addition, increased production has led to a scarcity of good production areas (Gullestad, 2011). To address these challenges, in 2015, a temporary licensing regime, *the development license regime,* was introduced. The intention was to stimulate technological innovations. As a result, several new production systems have been developed (Føre et al., 2022; MIF, 2004). Although promising, emerging technologies are characterized by *complexity, diversity, and uncertainty*, which challenge governing systems and create a pacing problem (Marchant, 2011; Marchant, 2020; Marchant et al., 2011). In Norwegian aquaculture, the pacing problem is driven, at least partially, by the development license regime, as regulations are not able to keep up with the technological development (Jensen, 2019). Using the development license regime as an example, we explore how government-initiated innovations drive technological innovation to the extent that regulatory regimes are not able to keep up, causing a pacing problem.

Materials and methods

The article uses an inductive, multimethod approach. A case study approach offers an in-depth understanding of the pacing problem in its real-life context (Yin, 2012). Grounded theory, using semi-structured interviews, provides a starting point for exploring the pacing problem (Glaser & Strauss, 1967), which is supplemented by document data and -analysis (Asdal & Reintertsen, 2020). Our approach is also abductive, as a continuous interaction between existing theories and empirical evidence generates hypotheses about the pacing problem (Timmermans & Tavory, 2012).

This study is a part of the DEVELOP project, financed by the Research Council of Norway (grant no. 301486).

Results

We study the development license projects in terms of their complexity, diversity, and uncertainty and how these characteristics challenge the governing system(s). First, development projects were required to be significant innovations and have therefore become complex assemblages of various technologies, e.g., oil platform technology combined with traditional aquaculture technology (Føre et al., 2022). As technological complexity increases, the complexity of regulatory processes increase, and become increasingly slow (Gaudet & Marchant, 2011). Second, to facilitate the development of a range of different technological solutions, development projects were required to differ significantly from each other. Without established guidelines, the resulting diversity proved to be a challenge for the bureaucracy in terms of processing and evaluating the development projects. Finally, complexity and diversity led to uncertainty, not only in terms of investment risk but also concerning the future trajectory of the technological solutions (Herkert, 2011), and the management thereof.

Conclusion

This article shows how government-initiated technological innovations lead to a pacing problem in Norwegian aquaculture governance. In particular, it shows how complexity, diversity, and uncertainty of the development projects, cause the governing system(s) to lag industry innovation. The question is whether initiatives, such as the development license projects, will be a solution to the perceived problems, or whether we are going down rabbit holes, in which the pursuit of one solution simply further exacerbates the pacing problem?

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AQUATIC BACTERIAL COMMUNITIES: FORCES AND DYNAMICS BEHIND THEIR FORMATION AND IMPACT IN EMBRYO AND LARVAL CULTURE

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Introduction

The majority of successful aquaculture has come from a relatively small number of species; however, still many economical important species remain underdeveloped. In particular, egg hatching and survival of larvae have proven to be bottlenecks to the complete and sustainable aquaculture of many species. The European eel is one such yet underdeveloped species, both as human food source and as object needing conservations measures after a dramatic decline in glass-eels (juveniles) over the last 40 years (Dekker, 2008). During their early life stages in the wild, they are exposed to oceanic environments low in microbial activity (Sjöstedt et al., 2014) while in aquaculture, they are vulnerable to bacterial infections (Sørensen et al., 2014) due to an immature adaptive immune system (Suzuki et al., 2000; Swain et al., 2009) well known especially for marine fish larvae (Hansen & Olafsen 1999). A stable and healthy microbial community within these aquaculture systems is therefore a prerequisite for a suitable European eel production environment. Eel therefore provide an excellent model for investigating the effects of microbial communities on fish egg hatching and larval survival. Aquaculture microbial community is significantly stabilized utilizing RAS technology (Recirculating Aquaculture Systems) (Attramadal et al. 2013). RAS enable stable microbial systems over time, by the constant feedback of nutrients released from organisms providing a selection and stabilization force of the microbial community (Attramadal et al. 2013). Although a well-balanced community is known to be of pivotal importance for the system, little is known regarding the forces that drive stability and how to reach a healthy state (Blancheton et al., 2013). By using microbiological and chemical analysis techniques, we compare pelagic eel embryonic and larval bacterial communities in response to water cleaning treatments such as ultra-filtration (500kd), UV and ozonation as well as biological maturation to create different types of aquatic environments. The aim is to understand the dynamics of aquatic microbial communities and the forces that drive them. This knowledge is of pivotal importance to enhance offspring survival during the early life stages of sensitive marine fish species. Using the European eel as a model species, we utilize state-of-the-art chemical and microbiological techniques, to endeavour scientific adaptability of complex aquatic microbial communities and aim ultimately to improve sustainability of aquaculture.

Material and methods

Harvested eggs of captive bred European eel were fertilized using prediluted milt and activated using artificial sea water based on reverse osmosis water and reef salt (Sørensen et al., 2016). Buoyant eggs were distributed into three 60L conical incubators connected to three types of sea water applied as flow-through with water inlet at the bottom of the tank. Incubators were held at 18ºC, at low light and constant supply of 0.2 μm filtered air. Three water masses used for incubation were treated as follows: i) preconditioned microbial matured seawater in RAS; ii) afore mentioned water type passing through cross flow Ultrafilter ~500KD, removing bacteria and vira; iii) first mentioned water mass passed through UV-C and Ozon. Each incubator was sampled daily to estimate egg density, bacterial load, fertilization, survival, hatch success, and subsequently larval survival to day 12. Egg associated bacteria we quantified at 0, 2, 4, 24, and 48 h post fertilization using enzymatic metabolic processes of bacteria (Bactiqant, Mycometer, Hørsholm, Denmark). Along with egg-associated bacteria, we quantified bacteria in the incubation water both regarding in- and out-flowing water to assess impact of eggs and their bacteria on water quality. Water characteristics of in- and out-let water were additionally analyzed by spectrophotometry characterizing the fluorescent dissolved organic matter (FDOM) known to nourish heterotrophic bacteria in the rearing medium. Hatch success was assessed by sampling ~100 buoyant embryos at 50 HPF from each water type, inserted into triplicate antibiotic flask containing 200 ml of the respective water but with the addition of 50 ppm rifampicin and 50 ppm ampicillin (Sørensen et al., 2014), and incubated in a temperature controlled environment (18ºC) until 60 HPF, when the ratio of hatched and unhatched larvae was determined. Larval survival was estimated by random selection of 3 × 20 larvae from each water type and inserted upon hatch into 200 ml flasks of antibiotic similar to above. Flasks with larvae were cultured in a temperature controlled environment and survival was quantified daily throughout the yolk sac period until day 12 DPH (Sørensen et al., 2016). Larval microbial community was assessed from larvae reared in kreisel tanks after hatch until day 10.

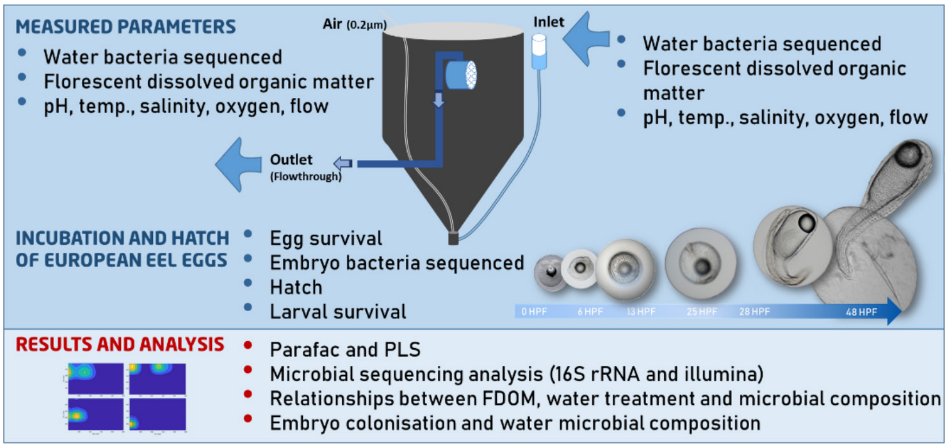

Fig. 1: Bacteria activity on incubated eggs and in the rearing water of European eel eggs reared using Matured, Ultra filtered (500kd crossflow membrane), and UV + ozon treated seawater.

Results and Discussion

The study outline links between microbial processes in a hatchery setup and how cultured organisms influence this at different means of water cleaning. Figure 1 outlines the measured parameters and how results and analysis are approached. We show how potential water cleaning techniques e.g. ozon and UV improves some rearing scenarios, while the associated instability that may follow negatively impacts other. The links between water biochemistry and emerging microbial community in water and colonizing embryos is discussed in light of survival.

Results show a steady and constant build-up of bacteria numbers on embryos but due to community differences resulting in different survival of embryos and larvae. Bacterial colonization happens rapidly and various filtration techniques can improve survival, but tend to induce instability and increase variability forming a “virgin” water mass ready for colonization by opportunistic pathogens (Vestrum et al., 2018). By analyzing water content and characteristics regarding fluorescent dissolved organic matter, we could differentiate between the carbon footprint of ‘good’ and less ‘good’ aquatic environments and their effect on colonization level/speed on incubated eggs.

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PROGRESS IN HATCHERY DEVELOPMENT FOR EUROPEAN EEL

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Introduction

European eel, *Anguilla anguilla*, is critically endangered and hatchery technology for commercial production of glass eels is fundamental to sustainable eel aquaculture, as wild-caught glass eels is still basis of eels cultured to marketable sizes. During the past 15 years, DTU researchers have engaged in the development of captive breeding technology for European eel aiming at a closed life cycle in culture (Tomkiewicz et al., 2019). As reproduction and early life history in nature is uncharted, experimental research is pre-requisite to fill gaps in knowledge. Thus, on the one hand research has targeted broodstock nutrition and reproduction, while on the other the establishment of larval culture systems, rearing conditions and diets. Encouraging for this research is the progress of hatchery technology for Japanese eel (*A. japonica*), now approaching commercialization. To date, European eel research has successively improved assisted reproduction methodologies leading to enhanced broodstock responsiveness, quantity, and quality of gametes as well as viability of offspring, now entering the first feeding stages. Broodstock involve captive-raised and wild-caught females using the latter as benchmark, while targeting a closed-cycle production, also aiming at benefits regarding lower disease risks and better performance in culture systems (low stress). Here, we present progress made during the innovation project ITS-EEL (2018-2021) focussed on up-scaling viable egg production and establishing the technology for feeding larval culture. This involved tailoring broodstock feeds and refining hormonal treatments, while progressing larval survival through custom-designed culture systems and novel diets for feeding larval culture.

Material and methods

The experimental research was conducted at the prototype eel hatchery, EEL-HATCH, Hirtshals, Denmark. Farmed broodstock were raised from the glass eel stage either at commercial farms or at the EEL-HATCH facility on customized feeds (SPAROS) for periods between 1 (Royal Danish Fish) and 3-4 years (EEL-HATCH). Wild-caught broodstock was obtained from a Danish nature reserve, Saltbaek Vig, through September and October new moon periods and transported to the EEL-HATCH facility. Here, broodstocks were acclimatized to 36 psu and 20°C at low intensity light:dark 12:12h photoperiod (Tomkiewicz et al., 2019), maintained in RAS setup. System maintenance involved water exchange of 5% per week, adjusted salinity to 36 PSU using Aquaforest Sea Salt (Brzesko, Poland). Different female broodstock treatments were tested including administration of estradiol for captive-raised females to synchronize ovarian development, pituitary extracts, CPE or SPE (Argent Chemical Laboratories, USA) at various levels (18.75, 20 and 25 mg/kg body weight), and different doses of 17α,20ß-dihydroxy-4-pregnen-3-one (Sigma-Aldrich, USA) to induce final maturation and ovulation, preceding strip spawning (Tomkiewicz et al., 2019). *In vitro* fertilization has been applied using pre-diluted milt at a fixed ration to newly stripped eggs, while being activated under standardized conditions (Butts et al., 2014). Buoyant eggs were incubated in 60L black incubators supplied filtered 36 PSU seawater and 0.2µm filtered air at 18°C in darkness. Following hatch, larvae were moved to custom made 70L semi-Kreisel-type tanks, applying stabile rearing conditions at 20°C and darkness until first feeding, happening in 8L Kreisel tanks at 18 PSU and 20°C.

Results and Discussion

Seven full reproduction series leading to larval production was conducted of where two series applied captive-raised females and five tested wild-caught. Diets for captive-raised female, targeted enhanced essential fatty acid composition, resulted in reproductive responsiveness similar to wild-caught silver-eels. Wild-caught females produced larger quantities of eggs of superior survival however once hatched, the larval quality of captive-raised approached that of their wild-caught counterparts (length and body area) (Kottmann et al., 2020a). Dietary improvements also enhanced male reproductive

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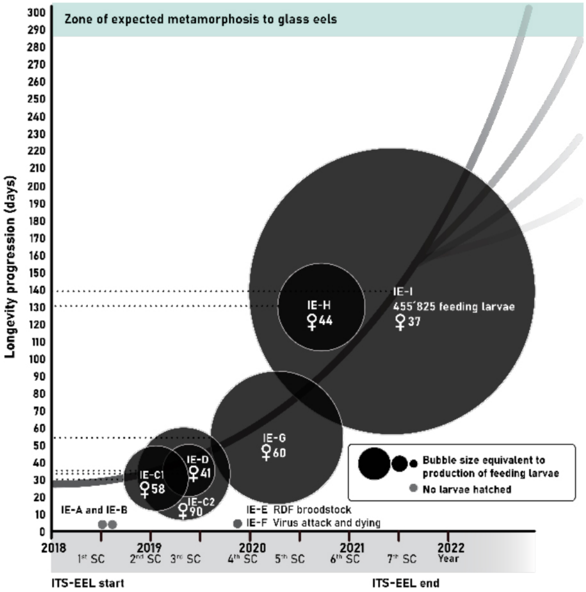

performance (Butts et al., 2020). Reproductive experiments documented that egg quality and embryos of SPE-treated females was higher than for CPE-treated females, however, the appearance of larvae surviving until the feeding stage was similar (Kottmann et al., 2020b; Benini et al., 2022). Here, treatment of 20 mg SPE/kg female body weight adjusted by the metabolic rate was best. Moreover, generation of dedicated tanks and RAS adjustments in rearing conditions facilitated high survival of embryos and yolk sac increasing numbers entering feeding and dietary trials. In total, 276 female eels successfully reproduced, with an avg. production of 447,333 to 672,865 eggs per female and mean fertilization success of 32 to 48% among series. This resulted in 21,167 to 70,217 hatched larvae per female, of which 2,191 to 12,320 larvae per female reached first feeding. Overall, an yearly average of ~30 M buoyant eggs was obtained, leading to an yearly hatch of ~3.5 M larvae of where ~0.5 M reached the feeding stages (10 dph). The costs of production currently entail prices of ~0.17 € per hatched larvae and correspondingly, the price per larvae reaching the first feeding stage is ~1.8 € incl. Notably, an economical analysis conducted shows a cost reduction of ~50%, once the present high level of research is no longer needed (i.e. when glass eel production and technology are established). These results, will be presented and discussed in context of an emerging hatchery enterprise.

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THE EFFECT OF SODIUM HYPOCHLORITE ON TOMONTS OF THE PARASITE *Amyloodinium ocellatum*

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Introduction

The parasite *Amyloodinium ocellatum* is one of the most common and important dinoflagellates infesting fish. *A. ocellatum* causes serious morbidity and mortality in both brackish and marine warmwater fishes farmed in aquaculture facilities worldwide. *A. ocellatum* has a direct, but triphasic life cycle that can be completed in less than a week in favourable environmental conditions, consisting of a free-living flagellate, the dinospore, the parasitic stage, the trophont, and a cyst stage, the tomont. Current therapies target the free-living flagellate and trophonts, while tomonts are highly resistant to treatments. More studies are needed to evaluate the efficacy of new treatments on tomonts to reduce the impact of this parasite on aquaculture.

Material and Methods

Tomonts were obtained from European seabass *Dicentrarchus labrax* infected experimentally with *A. ocellatum* into an *in vivo* propagation system on EPPO-IPMA. Infected fish were immersed on freshwater and tomonts were collect, washed, counted and exposed to a solution of sodium hypochlorite (SH; 13% chlorine) at 10, 100, 250, 500, 750 and 1000 ppm of chlorine and incubated on 96 flat bottom well-plate at 23ºC for 96h. Additional tests were performed by exposing tomonts for 1 h to SH with the same concentrations and washed before incubation. All tests were performed in triplicate, with a negative control. Evaluation of tomont inactivation was done by daily monitorization of sporulation (tomites/tomont) till 72 h. Tomonts that did not perform any division in the first 72 h were considered inactive. Dinospore production per tomont was also evaluated at 96 h of incubation.

Results and discussion

Continuous exposure to SH for 72 h showed to be effective in reducing tomont division at concentrations as low as 10 ppm, and to completely arrest the division process at 250 ppm (Figure 1A). Exposure for 1 h to the same concentrations revealed a similar trend, with 10 ppm being effective at limiting tomont division and 250 ppm arresting completely the division process (Figure 1B). Tomont inactivation was significantly higher after a continuous exposure to concentrations higher than 100 ppm ($p\text{-value} < 0.00001$) (Figure 1C) Similar results were observed for an exposure to the same concentrations for 1 h

Acknowledgments

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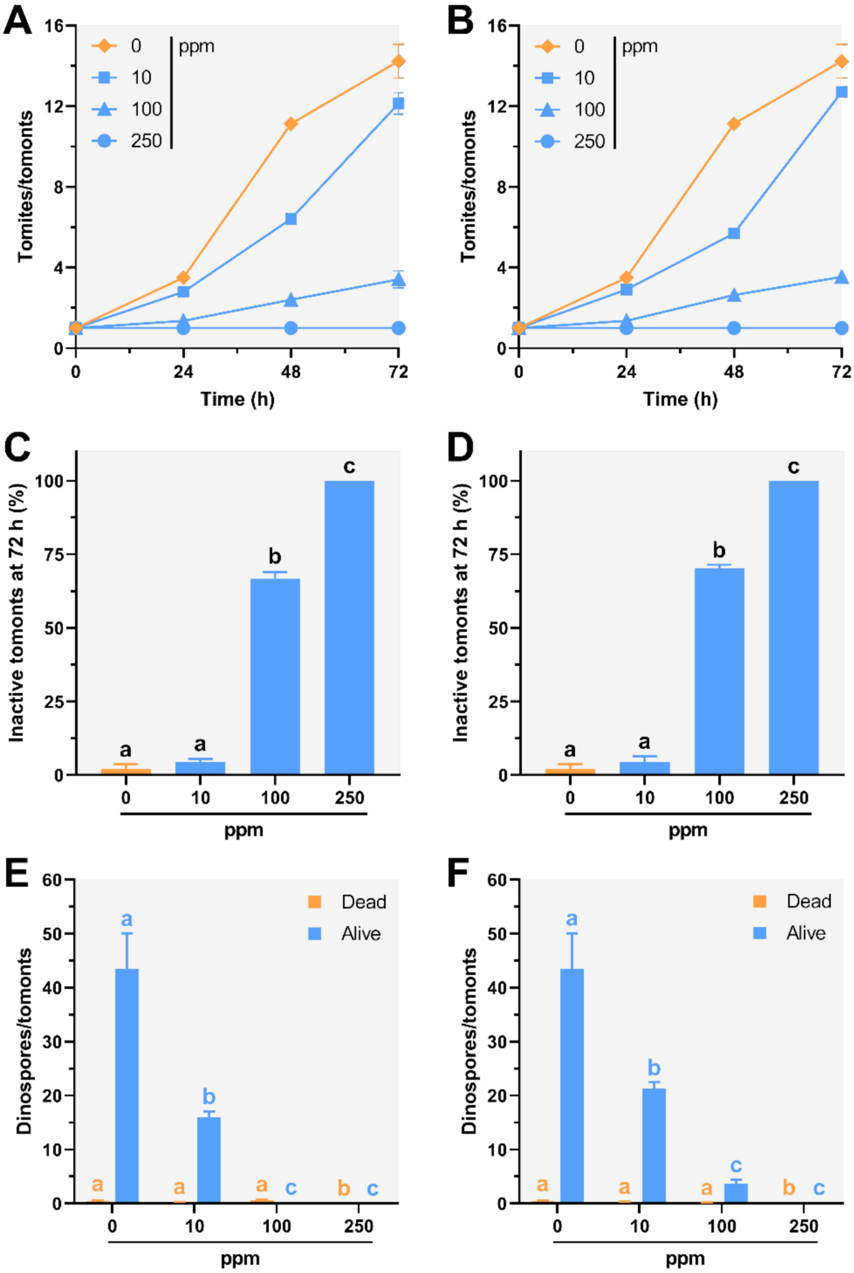

Figure 1. Inactivation of the parasite *Amyloodinium ocellatum* with sodium hypochlorite. A, B - Time course of tomont division after a continuous (A) and 1 h (B) exposure; C, D - Proportion of inactive tomonts after continuous (C) and 1 h (D) exposure; E, F - Dinospore production per tomont after continuous (E) and 1 h (F) exposure. Different letters indicate significant ($p < 0.05$) differences (one-way ANOVA with Tukey's post hoc test, n=3).

LESIONAL PATTERN OF TURBOT (*Scophthalmus maximus*) FURUNCULOSIS: NEW ADVANCES FOR INDUSTRIAL DIAGNOSIS

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Introduction

Turbot (*Scophthalmus maximus*) is a flatfish of great importance in European aquaculture (APROMAR, 2021). One of the biggest threats of turbot culture is furunculosis, a disease caused by the bacterium *Aeromonas salmonicida* subsp. *salmonicida* (Coscelli et al., 2014). In turbot, furunculosis mostly appears as a chronic disorder, where single to multiple swelling nodules emerge through the skin of the animal. These nodules are firm, grey to reddish, usually delineated by a rim of pale tissue and vary in size (2-6 mm in diameter) (Coscelli et al, 2014). However, the distribution pattern of nodules has not been thoroughly described and remains as a knowledge gap to the understanding and handling of the disease. Geographical Information Systems (GIS) are a software typically used in Earth sciences, but in the last few years, they have also appeared as a very useful tool for human and animal health sciences (Cromley, 2003), since they allow the treatment of different kind of data preserving their spatial component. Therefore, the objective of this work was to study the lesional pattern of turbot chronic furunculosis by means of GIS.

Materials and methods

For this study, 165 turbot coming from tanks naturally infected with *Aeromonas salmonicida* subsp. *salmonicida*, that showed skin lesions compatible with furunculosis, were selected. The number of lesions and their location (ocular or blind side) were recorded, and skin samples were taken for histological and immunohistochemical confirmation of the disease. Two photographs (one per side) of each animal were taken, besides recording of their age, weight, and time elapsed since diagnosis. Images were later digitalized, and lesions were transferred to a unique standardized template and processed with ArcGIS© software.

Results

The histological features of the skin samples where compatible to those described in the literature for turbot chronic furunculosis (Coscelli et al., 2014). Immunohistochemical studies confirmed the presence of *Aeromonas salmonicida* antigens in most of the samples taken.

The mean number of lesions per fish was 2.8 ± 0.3, with bilateral asymmetry with predominancy of the ocular side (2 ± 0.2 mean lesions), when compared to blind side (0.7 ± 0.2). Furthermore, as it can be noted in figure 1, most fish had lesions on ocular side, with only 3% of them showing lesions affecting the blind side alone.

With Kernel density estimation tool from ArcGIS© software, it was possible to project a heatmap with the most frequent occurrence sites for furunculosis lesions, such as the mouth and the area of the head rostral to the operculum, in both ocular and blind side (figure 2). In addition to these findings, variations were seen depending on the size of the fish, with lesion concentration in the rostral edge of the operculum on turbot with lower weights and in the mouth on fish with the higher ones.

Discussion

The evidence of a clear distribution pattern in turbot furunculosis lesions entail a new step in this disease management. Operators at turbot culture facilities can easily be trained to inspect the heat regions detected in this study, to quickly detect the disease once it appears on the farm, preventing big outbreaks. Moreover, since most affected fish show lesions in the ocular side, it is possible to detect many of them in the conveyor belt of the grading line, saving the time of turning them over and making viable the development of diagnostic techniques based on gross examination and automatized detection and discarding of affected animals.

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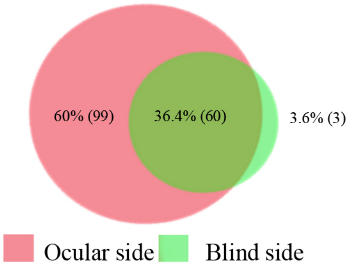

Figure 1 Venn´s diagram showing fish with lesions affecting the ocular side and the blind side (n=165).

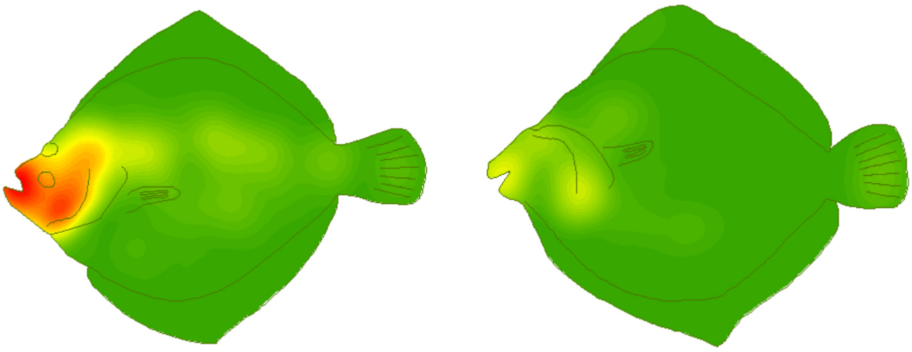

Figure 2 Kernel density estimate for the prevalence of furunculosis dermal lesions, red colour corresponds to the highest density, while green corresponds to the lowest (n=165).

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SIZE MATTERS: A SOLUTION FOR HISTOLOGICAL PROCESSING OF SMALL SAMPLES

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Introduction

The use of zebrafish as a model organism has grown exponentially in recent years. This small organism creates a point of union between two great fields of research: aquaculture and biomedicine. Being able to apply the technologies and tools of one field to the other improves the knowledge of both disciplines. Several research uses embryos or larvae of just a few hours or few days of life, for example, for testing molecules with immunostimulatory effect for their application in diets. The study of these models in early stages of development provides interesting advantages for many laboratory techniques. However, the small size of these fish encourages them to be lost or spoiled during the process, as it happens with histological processing. Therefore, it is interesting to look for alternatives that allow the paraffin-wax embedding of samples of such a small size. The objective of this work has been to develop an inclusion protocol focused on zebrafish embryos and larvae.

Materials and methods

The experience in the processing of histological samples, with the knowledge of the behaviour of the agarose against different compounds, and the learning of new technologies such as 3D print were added to achieve the objective. The methodology followed is based on the design and printing of a rectangular countermold of acid polylactide (Fig 1). Its surface has protrusions with custom shape and dimension, according to the stage of development of the fish to be studied. From this one countermold, an agarose mold is obtained with wells corresponding to the protrusions of the printed countermold.

Results

The agarose mold allowed the embedding of up to 16 zebrafish embryos 48 hours post fertilization. In addition, the design of the mold and the wells includes two important aspects: maintain the traceability of the samples, since the wells are individually identifiable, and allow the control over the orientation of the embryo, which may be important for certain studies. Furthermore, this procedure allows the treatment of multiple specimens as if they were a single sample, enabling its histological processing by routinary means.

Conclusion

This solution is another example of how multidisciplinarity within the work teams is a strong ally for the improvement of the techniques applied to this model species and other small size samples. Being able to design and manufacture this type of molds in the laboratory opens the door to endless potential applications.

Acknowledgments

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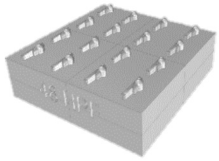

Fig 1 Design of rectangular countermold of acid polylactide.

HYBRIDIZATION AND TRIPLOIDIZATION AS TOOLS TO IMPROVE LARVICULTURE IN PIKEPERCH *Sander lucioperca*

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Introduction

Pikeperch (*Sander lucioperca*) farming has been studied extensively in last couple of decades, nevertheless, the progress is still slow. One of the main bottlenecks is still the variable outcome of juvenile production (Policar et al., 2019). According to Müller et al. (2017) hybridization with Volga pikeperch (*Sander volgensis*) may result in an upturn of favorable properties in production, such as straightforward weaning to the dry feed, and more efficient start of first exogenous feeding as well as the reduction of cannibalism which could improve the rearing in all types of production systems of pikeperch. Triploidization as a method to improve production has already found its application in aquaculture. It is now commonly used in salmonid fish species with the main aim to avoid sexual maturation of animals and thus improve meat quality and growth (Janhunen et al. 2019). Likewise, the existence of a third set of chromosomes increases the chance that recombination of genes will avoid population depression (Fraser et al. 2012). Triploidization might reduce the aggressiveness of the fish (Garner et al. 2008) what might be beneficial in pikeperch considering its cannibalistic behavior in young juvenile stage (Ljubobratović et al., 2015; Colchen et al., 2020) The aim of the present study was to evaluate the effects of hybridization and triploidization on pikeperch larviculture.

Material and Methods

The eggs used in the present trials were obtained via artificial reproduction of six female pikeperch in preseason according to protocol described by Ljubobratović et *al. (*2021). The eggs of three females were fertilized with the sperm of pikeperch and the rest with sperm of Volga pikeperch. Half of the eggs of each group was submitted to the triplodization induction via hydrostatic pressure shock as described by Káldy et al. *(2021*). Following hatching all larvae from each group were mixed. At 6 days post-hatch, larvae were stocked in a larviculture recirculation system. Thus, in total four duplicate groups were formed: diploid (D), triploid (T), hybrid diploid (HD), hybrid triploid (HT). The trial lasted 40 days, in a temperature range of 16 ± 0.5 ºC and a light regime of 14:10 L:D. Larvae were fed with freshly hatched and enriched *Artemia nauplii* and starter feeds, finally weaned at 32 DPH. Sample measurement of body weight and length were performed on a weekly basis while all larvae were counted at the end of the trial and evaluated for the presence of swim bladder inflation.

Results

Chromosome analysis showed that the triploids were free of the presence of mosaic structures in the sample. Condition factor was significantly affected by triploidization being lower in triploids. Juvenile yield in form of swim bladder inflated juveniles per volume of rearing unit was significantly affected by hibridization. Likewise, hybridization had a significant effect on the percentage of swim bladder inflated fish.

Discussion

Hybrid groups showed enhanced larviculture performance in terms of production of fish with inflated swim bladder. With respect that the larviculture survival is the main bottleneck for the upgrade of pikeperch farming, this improvement might be beneficial for the sector and thus further evaluated in future studies. Triploidization did not yield positive results in in this study, however should be evaluated in more appropriate nutritional regimes.

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Acknowledgements
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IMPORTANCE AND FINDINGS OF GERMAN NEARSHORE TEST SITE IN THE MULTI-USE OFFSHORE PROJEKT UNITED

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Within the research project UNTIED (Multi-Use offshore platforms demoNstrators for boostIng cost-effecTive and Eco-friendly proDuction in sustainable marine activities), five different pilot sites in Germany, the Netherlands, Denmark, Belgium and Greece were chosen to investigate current bottlenecks relating to the large-scale installation of ocean multi-use activities, demonstrate business synergies and benefits of ocean multi-use and provide a roadmap for deployment in future multi-use sites and potential scaling barriers to be addressed through best practices and lessons learnt. Each Pilot investigates different aspects. Focus within this work is given to the findings and importance of the nearshore site of the German pilot in Kiel. The German pilot as a whole looks into possible synergies of the cultivation of blue mussels, seaweed and offshore wind energy. Main purpose of the nearshore site was testing of materials, antifouling strategies, sensor connections and data transmission, handling of equipment and training of staff in a small workin mussel and algae open water aquaculture. Testing of material revealed weaknesses in camera fixtures and lead to improved design and material usage.Testing of antifouling strategies for stationary objects with air diffusers, wipers, UV light, ultrasonic vibrations, novel mesh covering and biological friendly chemical antifouling coating showed best result for the wiper technique and the chemical coating. Testing of sensor connectivity and transmission in the nearshore site allowed for adjustment and fine-tuning without costly repetitive offshore trips. Training of the staff in deploying handling and adjusting equipment in a comparably low risk, easily assessable surrounding allowed for repetitions and adjustments in an easy way. As the nearshore site lies in the Baltic Sea, while the offshore site lies in the North Sea mussel and algae growth, recruitment and settling was not tested, as due to the different conditions a transferability and value of knowledge was deemed highly unlikely. However if materials, techniques and data-transmission and handling does fail in a low risk, low energy nearshore site, they will not work in the high energy high risk offshore locations. Yet findings of the nearshore site have to be critically checked.

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A BIOENERGETIC APPROACH TO MODELLING OXYGEN AVAILABILITY IN FISH CAGES. TOWARDS A TOOL FOR EARLY WARNING IN AQUACULTURE

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Introduction

Oxygen constitutes one of the most important environmental factors for fish farming since it affects fish growth and health. Given that the sustainable development of aquaculture necessitates the adoption of precise and automated methods, it is important to develop tools that enhance our predictive as well as monitoring capacity with respect to the oxygen conditions at the farming sites (Royer et al., 2021). In this study we propose a modelling approach that uses bionergetic models coupled with the water properties at the farming site to simulate the oxygen consumption of fish and the oxygen availability within a typical production unit (i.e. marine cage). The aim of constructing such models is to incorporate them to an early warning system that will be able to utilize remote sensing data and produce relevant alerts to the operators of aquaculture facilities in Greece.

Materials and methods

Models based on Dynamic Energy Budget (DEB) theory (Kooijman, 2010) previously developed for European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), and meagre (*Argyrosomus regius*) (Stavrakidis-Zachou et al., 2019; 2021) were validated against production datasets with respect to their predictions of growth, feed consumption and oxygen uptake. These models simulate the bioenergetics of individual fish as a function of temperature and food availability following with an extrapolation to the population level. Therefore, the models can be used in simulations to predict and report the total oxygen demands of a fish cage under the prevailing environmental conditions. In addition, we developed an approach to assess the adequacy of oxygen availability by modelling the supply of oxygen to the cage for a given area as a function of water velocity and cage characteristics. The above approach was conceptually tested at HCMR's pilot scale farm (Souda Bay, Greece).

Results

Validation of the models showed that they performed well and could sufficiently capture changes in oxygen uptake under varying environmental conditions. Subsequent application of the models at the pilot scale farm under typical farming conditions revealed that the models could not only predict the anticipated oxygen availability patterns but also detect potentially alarming signals for the farm operation. Specifically, using the E. seabass as an example, it was observed that the most frequent for the region water velocities (2-6 cm s^{-1}) provide sufficient oxygenation at the cage level since the oxygen supply far outweighs the oxygen demands of the fish (Fig 1). However, particularly low water velocities, which have however been recorded in the area (0.2 cm s^{-1}), may lead to suboptimal or even dangerous oxygen conditions for the fish.

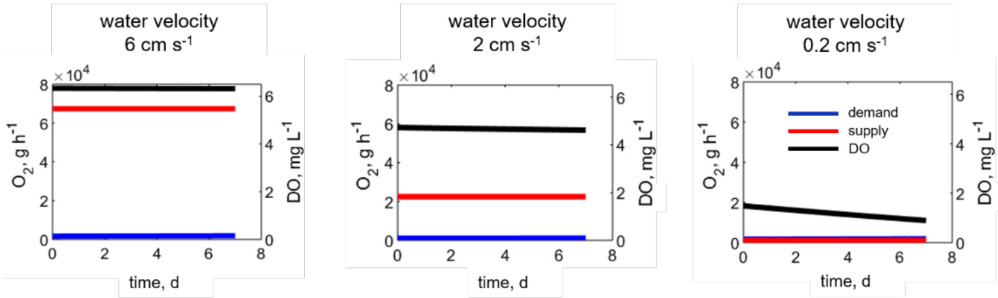

Fig. 1. Simulations of E. seabass juveniles (50g) reared at the pilot scale farm at Souda under three water velocities (6, 2, 0.1 cm s^{-1}). Red indicates the supplied oxygen in the cage, blue the oxygen demand by the fish, and black the dissolved oxygen in the cage. Temperature: 27°C, stocking density: 10 kg m^{-3}.

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Conclusion

In this study we used mechanistic models for three fish species, which based on bioenergetics, simulate the oxygen consumption of a fish population at the marine cage level. This output is integrated with the estimation of oxygen supply in the area in order to assess the oxygen available to the fish within the production unit. At a subsequent stage, the simulated output will be fed to an early warning system to provide alerts to aquaculture farm operators in cases of low oxygen availability. Initial testing of this approach has validated its capacity in predicting anticipated patterns and highlighted its potential as a tool for detecting alarming scenarios.

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NORTHERN EUROPE'S SUITABILITY FOR FLAT OYSTER (*Ostrea edulis*) REEF RESTORATION AND OFFSHORE AQUACULTURE

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Introduction

After centuries of overexploitation, flat oyster (*Ostrea edulis*) habitats are being protected and large-scale oyster reef restoration projects are about to be kickstarted in the North Sea, the English Channel and the north-eastern Atlantic. Oyster reefs are recognised biodiversity hotspots that provide many ecosystem services and bolstering of these habitats will provide ecosystem services that will support the marine ecosystems in northern Europe. At the same time, interest grows for offshore production of flat oysters, possibly combined with other marine uses such as offshore wind. Understanding the habitat requirements of flat oysters is key in the site selection process of restoration efforts and offshore aquaculture.

Material and methods

Establishing the theoretical niche for all life stages of the European flat oysters is done through a dynamic energy budget model forced with temperature, chlorophyll a and salinity in combination with sediment composition. The suitability of Northern Europe's marine environment for flat oyster is evaluated by applying the proposed niche model on a spatial scale and evaluating spatio-temporal variability in life history traits and metabolic characteristics such as fitness, reproductive output, settlement success, and growth.

Results

According to the results of this study, habitat suitability is limited by low maximum summer temperature and low food availability at higher latitude and in areas under strong influence of the Atlantic Ocean. Restoration efforts and aquaculture in Ireland and Scotland should be limited to protected and shallow coastal environments. Suitability in the southern North Sea is patchy and mainly depends on seabed substrate (for restoration and bottom culture). The coastlines enclosing the English Channel are highly suitable. The suitability of coastal areas is mainly limited by low salinity levels due to river runoff and unfavourable sediment.

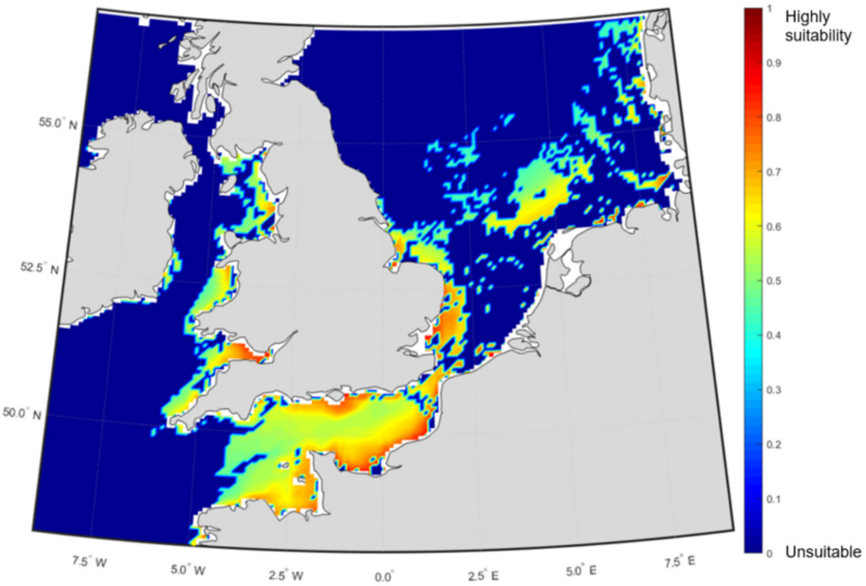

Figure: Site suitability for European flat oyster (*Ostrea edulis*), include locations that support high fitness, growth rate and reproductive output and enable life history events such as spawning and settlement (on suitable substrate).

COMPARING ASC CERTIFIED SHRIMP FARMS WITH CONVENTIONAL FARMS AND LITERATURE – SHORTCOMINGS AND OPPORTUNITIES

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Introduction

The Aquaculture Stewardship Council (ASC) is an independent not for profit organisation founded in 2010 by the World Wide Fund for Nature (WWF) and the Dutch Sustainable Trade Initiative (IDH). The ultimate goal of all ASC standards is to reduce the environmental and social impact of aquaculture worldwide. The standards are reviewed and revised every 3 – 5 years at a minimum in order to incorporate learning from stakeholders' feedback and, among other things, update the metrics within the standards according to the ASC Metrics Methodology.

The ASC Shrimp Standard was recently reviewed and data from certified and non-certified farms as well as from literature was compared and used to update the metric requirements. Three of the key performance metrics for shrimp production are farm survival rate, feed conversion ratio (FCR) and forage fish dependency ratio, which describes the quantity of wild fish used per quantity of cultured fish produced. These metrics not only provide measures of production efficiency, but are also important indicators of impact of aquaculture on wild fish stocks and the environment, reliance on limited resources, and fish welfare.

This data collection provides an interesting overview over shrimp production worldwide and shows what ASC can accomplish, but also where there are shortcomings especially in regards to data collection and comparability.

Methods

Within the revision of the ASC Shrimp Standard data from ASC certified and non-certified farms as well as literature data was taken into account. Survival rate, FCR and FFDR were selected for the purpose of this report to compare the performance of farms producing *P. vannamei* and *P. monodon*. Data on survival is differentiated by systems, based on the definitions used in the current ASC Shrimp Standard. The data was collected alongside additional metrics to inform the updating of the ASC Shrimp Standard. Data from ASC certified farms was extracted from a total of 233 audit reports (initial audits, surveillance audits and recertification audits) from 15 countries. Data from non-certified farms was provided to the ASC during the public consultation phase from a total of 257 shrimp farms, mainly covering Indonesia, Ecuador, and Thailand. Literature data was derived from a total of 24 scientific articles published until December 2019.

Comparisons between certified farms, non-certified farms, and literature values were made on the basis of averages and the standard deviation. Limited datasets and varying methods of data collection did not allow for statistical testing of the observed differences.

Table 1 Summary of data from literature and certified and non-certified farms, including quartiles

Requirement	Certified Farms	Non-certified Farms	Literature
Annual average farm survival			
Unfed and non-permanently aerated	33.4 ± 14.4	14.5 ± 8.8	no data
Fed but non-permanently aerated	62.9 ± 12.2	29.7 ± 19.6	60.3 ± 14.1
Fed and permanently aerated	78.3 ± 9.7	62.1 ± 15.5	81.8 ± 15.9
Forage Fish Dependency Ratio (FFDR)			
P. vannamei	0.9 ± 0.4	1.2 ± 0.6	1.4 ± 0.6
P. monodon	1.6 ± 0.4	1.6 ± 0.3	2.3 ± 1.3
eFCR			
P. vannamei	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.3
P. monodon	1.7 ± 0.1	1.5 ± 0.3	1.5 ± 0.8

(Continued on next page)

Results and Discussion

Results for annual average farm survival, FCR and FFDR are shown in Table 1. The data shows that survival rate seems to be an issue for non-certified farms. This was also mentioned in the stakeholder feedback as a major concern for farmers. Literature data shows significantly higher survival rates. This does not only underline the differences between research data (often conducted in closed laboratories) but also the importance to collect real time data on the farms. The Forage Fish Dependency Ratio (FFDR) describes the quantity of wild fish used per quantity of cultured fish produced. Fishmeal derived from by products that meet the sustainability and traceability criteria defined by the ASC Shrimp Standard are not included in this calculation. Values for non-certified farms were estimated based on an average fishmeal content of 20%, as this was stated as average by the farmers. Most scientific literature provided detailed information on fishmeal content in the diet. If this was not available, the average FM content reported by the FAO of 25% was used. FFDR values for *P. vannamei* are lower on average for certified farms, whereas values for *P. monodon* are similar for farms and show high fluctuations in literature. This could be due to the fact that some certified farms deduct the sustainably sourced FM, whereas there was no information available for non-certified farms or literature. The general decreasing trend of reliance on whole fish from wild stocks in recently collected data (on farms) relative to older data from literature could also be an explanation. These differences and uncertainties underline the importance of field studies on non-certified farms in order to evaluate the difference between certified and non-certified farms in more detail.

Certified farms had a slightly higher FCR than non-certified farms for both species considered. This could be a result of differences in data reporting methodology, or could reflect a potential trade-off between feed efficiency and changing feed composition to include lower rates of fish meal and fish oil.

This short data overview shows the variability between research and farming, underlining the importance to collect data in field studies. However, most of the data was reported to the ASC and not collected specifically for this purpose. Different methods can have a tremendous effect on the quality of the data. In the future, the ASC will need to conduct more field verification studies and thus work closer with both academia and farmers all around the globe.

APPLICATION OF EPIDEMIOLOGICAL MODELLING *OF Sparicotyle chrysophrii* TRANSMISSION IN *Sparus aurata* FARMS

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Introduction

Mediterranean aquaculture production is known to have impacts on the environmental and marine habitat, impairing water and bottom sediment quality. Furthermore, rapid development of cage fish farming has been associated with propagation of infectious diseases, pathogens and parasites.

Sparicotyle chrysophrii is a common parasite of cultured Gilthead sea bream (*Sparus aurata),* an important species for Mediterranean aquaculture production. The parasite attaches on fish gills and can cause lethal epizootics in sea cages. Infections depend on environmental factors, in particular on water temperature (Antonelli et al., 2010).
Epidemiological modeling represents a valid tool to help fish farmers understand parasite transmission and evaluate possible control measures. To this purpose, in the following we analyze a novel epidemiological model of *S. chrysophrii* transmission.

Methods

We developed a novel stratified compartmental model where each compartment X_{ja} represents the abundance of fish infected with j juvenile parasites and a adults. The model further accounts for environmental abundance of eggs and miracidia, where the latter determines the force of infection, i.e. the rate at which parasite larvae attach to fish. The dynamics further accounts for egg production and hatching, parasite development, baseline mortality for parasites and fish, and parasite-induced fish mortality. Critical parameters controlling the progression of the disease are assumed to be temperature dependent.

We applied the model to data collected in six cages of a sea bream farm managed by Cromaris (Bisage, Croatia). A controlled experiment was run between February and November 2021 in which 30 fish were collected each month and, for each fish, all eight arc gills were examined to count the number of attached parasites.

We estimated model parameters in a Bayesyan framework, sampling the posterior distribution with a Markov Chain Monte Carlo algorithm. Specifically, the model estimates the temporal dynamics of the probability that a sampled fish hosts a certain number of adult parasites. The likelihood of a monthly sample is calculated assuming a multinomial distribution, where the event probabilities are estimated through the deterministic model simulation given a certain parameter set. We further included the total number of fish alive at any time in the determination of the likelihood.

Results

Results show that the model is able to reproduce the distribution of the number of parasites hosted by the sampled fish (Figure 1), as well as the temporal progression of the prevalence of infection (i.e., the number of fish hosting at least one parasite). Data shows that prevalence has a peak in August, after the period with the warmest water temperature, and then decreases.

Discussion and conclusions

We applied a novel epidemiological model to a dataset of *S. chrysophrii* infections collected in a sea bream farm. The model succeeds in simulating the effects of environmental factors (e.g., water temperature) on the infection dynamics. Posterior distribution of parameters shed insights on crucial process rates that control disease transmission. Future developments could involve coupling the epidemiological model with a fish growth model to simulate the effect of parasite burden on fish metabolism. The knowledge gained through this combined experimental and modeling exercise could be used in the future for designing and implementing strategies to control the spreading of the infection and, accordingly, to improve both fish health and aquaculture production.

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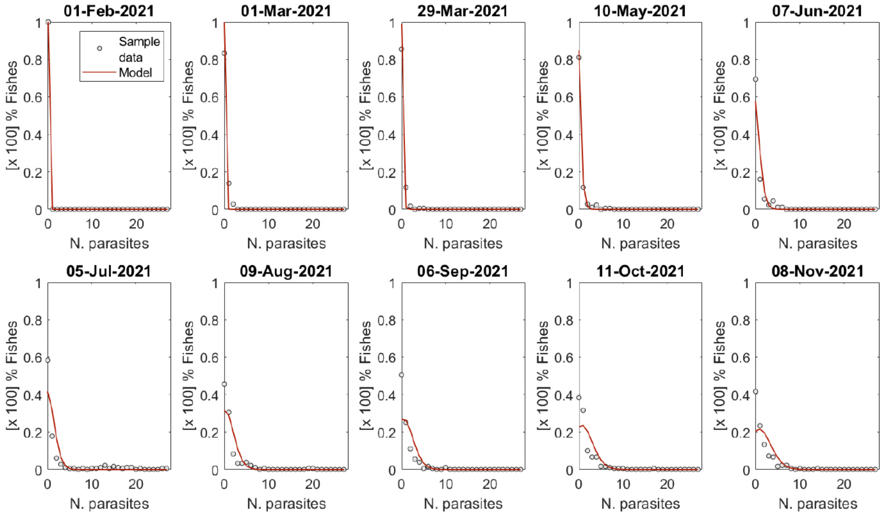

Figure 1. Parasite distribution within sampled fish. Gray circles are the sampled data, while the red line represents model outputs.

Acknowledgements

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AIR BASED CO2 MEASUREMENTS FOR INDUSTRIAL 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 fan, pump, and blower performance to transport the high CO_2 air more economically (save energy) out of the production facility. Dissolved CO_2 sensors have their limitations (low accuracy, pression and response time). However, it is possible to measure CO_2 directly in the outflowing air of the degassing unit. The air-based measurements can be a proxy for degassing performance. Air based non-dispersive infrared gas analysers are much faster, precise, and reliable and perhaps a good online control option for aquaculture CO_2 degassing units.

Material and methods

CO_2 removal in brackish and seawater RAS is theoretically less efficient compared to freshwater. We compared the degassing performance in fresh (0 ppK) and brackish water (12 ppK) at the Nofima Centre for Recirculation in Aquaculture (NCRA), Sunndalsøra on the main experimental degassing units (AquaOptima AS, Norway). A $CO_{2/}$Air water vapor compensated CO_2 meter (Li-850; LI-COR Biosciences, United States) was verified at the in- and out-flow of the degassing tower with high precision automated titration water-based carbonate data (TL7000; VWR International AS, Germany) and dissolved CO_2 measurements (OxyGuard International, Denmark). Dissolved CO_2 measurements (Oxyguard & TL7000) were done before the biofilter, after the biofilter and after the degassing tower. The LI-COR system (up to 2 measurements per sec) could provide fast, precise, and robust measurements in the air leaving the degassing units. Degasser design is system-specific and can vary in hydraulic loading rate, packing height and Gas:Liquid ratio. Therefore, degassed air CO_2 concentrations "needs to be calibrated" to the dissolved CO_2 levels in the water as a proxy for degassing performance (system-specific).

Results

As expected, removal of dissolved CO_2 from water and CO_2 increases in air leaving the degasser were proportional and no differences between salinities could be observed. In our system, "removing (delta- in and out- flow)" 1 mg L^{-1} dissolved CO_2 in water, increased air CO_2 "removal (delta out- and in- flow)" by about 52 µatm. Also as expected, CO_2 removal increased with an "absolute" increasing dissolved water CO_2 levels into the degasser from the fish tanks. In our system, an increase of 1 mg L^{-1} dissolved CO_2 out of the fish tanks into the degasser increased the "removal" CO_2 concentrations" in air by about 29 µatm. The air "removal (delta out- and in- flow)" values can be used to predict the incoming concentration of dissolved CO_2 into the degassing unit.

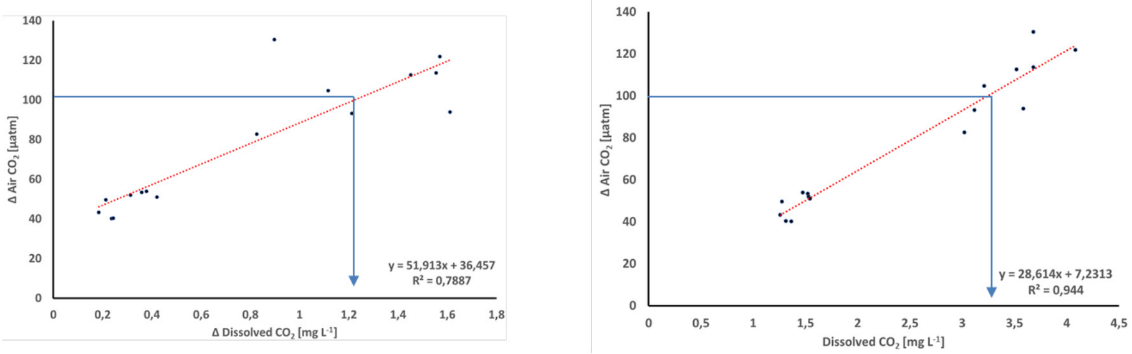

Figure 1: (A) Proportionality of CO_2 removal in water to air and (B) dissolved CO_2 concentration dependency of air CO_2 removal.

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Example

Under constant operating conditions, we observe an increase of 100 µatm in our off-gas. Using Figure 1 A, this corresponds to a CO_2 removal of about 1.2 mg L^{-1} in water. Using Figure 1 B, we estimate that our CO_2 concentration in water entering the degasser is about 3.2 mg L^{-1}. The calculated CO_2 removal rate is about 36% which fit perfectly with the manual measured data.

Discussion

Air based measurements showed a good correlation with dissolved CO_2 removal from water and allowed estimating dissolved CO_2 concentrations entering the degasser. Having online measurements in milliseconds of CO_2 concentrations in air could make the online LICORE unit reliable, and fast enough (which is a huge benefit compared to submerged dissolved CO_2 sensors) for controlling the degassing unit (blowers and pumps). In future automated CO_2 degassing unit controlled by online CO_2 measurements can likely contribute to energy savings in RAS production.

ALLOCATION OF ZONES FOR AQUACULTURE IN TUSCANY REGION: THE CASE STUDY OF FOLLONICA GULF

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Introduction

A key bottleneck of marine aquaculture in Italy has been due to the lack of allocated space. This is recognized to be one of the most relevant constraints to limit the expansion of the mariculture industry (COM 2021/236 final). The process through which space is allocated to marine farms, Maritime Spatial Planning, has been defined by ISPRA and the Ministry of Agriculture through a technical manual (Marino et al 2020). Such process, although not yet mandatory, is applied by competent authorities to help identify Allocated Zones for Aquaculture (AZA), consequently allowing for the culture of specified fish and bivalve species, and the monitoring of environmental impacts within such zones.

The first fish farms cluster in Italy was established by the Municipality of Piombino within the gulf of Follonica, allocating an extensive area to multiple mariculture activities.

The area, as we know it today, has been established through the administrative decision Delibera Giunta comunale di Piombino n. 104 del 17/3/2013, a practice lacking a true scientific-based zoning process. Due to an extensive expansion of aquaculture within this zone from 2009 to 2018, this has created a hotspot of fish and bivalve culture, all one next to the other.

Materials and methods

The whole licensed AZA within the gulf is more than 16 mil m^2 wide; allocating 5.8 mil m^2 to fish culture and 3.7 mil m^2 to bivalve culture. The remaining 7.1 mil m^2 were identified by the municipality as safety and transition zones.

The boundaries of the zone run due north-south and east-west. $\Delta\omega$ 2', ie 3.7km, $\Delta\lambda$ 3.2', ie 4.5km.

Isobaths run along the coastline (West to South-East), with depths ranging 20 to 35 m. On the Northern part of the AZA the seabed consists of dead mattes of *P. oceanica* (approx. 20m), whilst more intermediate depths are characterized by a mud and silt composition. The deeper ranges of the seabed are composed of sand and mud.

Hydrodynamic forces guarantee optimal water circulation in the bay; currents are seldom less than 10cm/sec, and with wind driven swell occurring within the 1st, 2nd, and more predominantly 4th quadrant (Fig. 1).

Data on aquaculture facilities, such as number of farms, size, production data etc. were collected having direct field survey with fish and mussel farmers operating in the AZA (Table 1).

Results and discussion

The present study identifies the administrative process and technical decisions which have led to the current allocation and use of marine space within the bay.

Each concession license within the AZA has been classified and analyzed in terms of: distance from ports, bathymetry, interference with fishing and tourism related activities, as well as production potential.

A comparison between actual and potential or desired production scenarios has been carried out to estimate and forecast the potential environmental impact and carrying capacity of the AZA.

Dependent on the production output and site-specific parameters (depth, sediment composition, and hydrodynamic forces) for each farm, an environmental monitoring scheme has been developed in correspondence to the ISPRA, ARPAT, and ASC Guidelines.

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	Grids	Cages	Diametre (mt)	Site depth (mt)	Net depth (m)	Production 2022 (ton)
Farm 1	3	25	22	24	10	650
Farm 2	3	24	25	30	10	1150
F 2 (new)	2	16	32	30	10	1100
Farm 3	2	20	30	35	10	1200
F 3 (new)	2	24	30	35	10	1400
Farm 4	3	30	28	22	15	1500
Total	15	142				7000

Table 1 Aquaculture facilities data in the Follonica Gulf (2022)

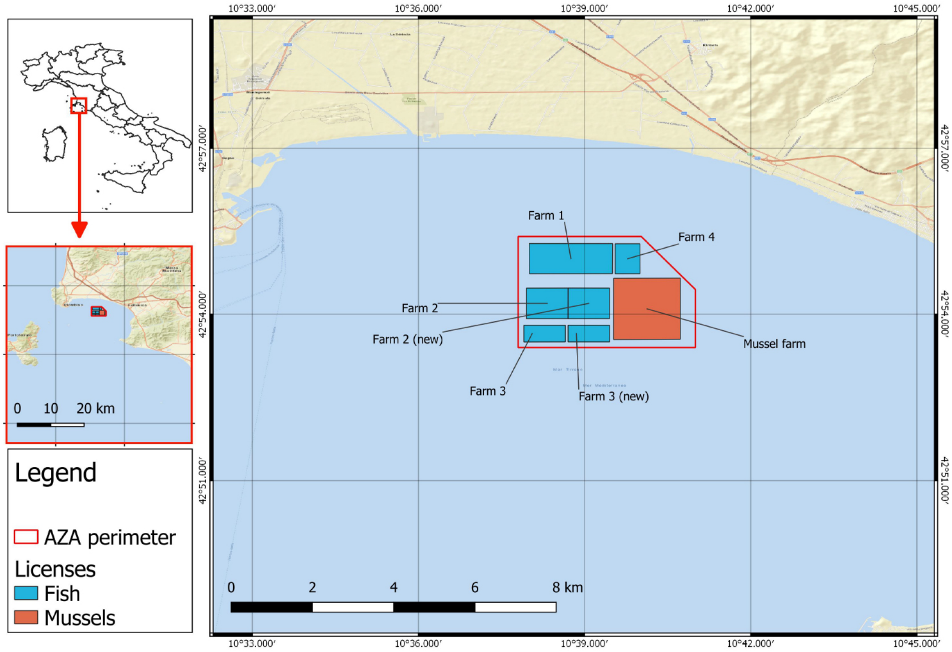

Fig. 2 The AZA in Follonica Gulf and the licenses for fish and mussel aquaculture

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BIOSECURITY IN SMOLT HANDLING. RESULTS FROM A STUDY OF PROCEDURES IN NORWEGIAN FISHFARM AND WELLBOAT COMPANIES

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Introduction and research question

Biosecurity is crucial for sustainable and safe aquaculture. Biosecurity entails a set of management measures to reduce the risk of transmission, development and spread of infectious diseases, between populations, production zones, means of transport, sites, and enterprises (Lillehaug et al. 2015). Deviations in biosecurity can be caused by limited knowledge, cost considerations, technology design, and increased professional and operational complexity (Larsen et al. 2020).

In Norwegian salmon farming, the smolt is essential. Actors within research, industry and government have urged for more knowledge about biosecurity, particularly for the smolt. A recent Norwegian study shows a great potential for infection control in the hatchery and wellboat operations, due to uncertainty associated with water quality, technical design and quality control (Larsen et al. 2020).

To improve biosecurity for the smolt, this research project will suggest measures for predictable and efficient water treatment and disinfection in RAS facilities (“recirculating aquaculture systems”) and smolt transport. The main study topics are microflora, technology, and operational routines. To find adequate measures, several methods will be employed, and the current presentation will report the results of the first step, namely a document analysis.

Method

This is an analysis of documents from the quality management systems of three Norwegian fishfarm and wellboat companies. Documents relevant for biosecurity have been selected, such as risk considerations, technical parameter settings, operational procedures, deviation reports, internal audits, shift schedules, etc. This document analysis is a supplement to the project’s main methods of microorganism sampling, analysis of technology design, and interviews and operations of operational routines. The presentation may give hints to tentative results from the other approaches too.

Background and earlier research

The transfer of microorganisms and infectious agents between fish populations and between systems has been a challenge for the aquaculture industry since its commercialization in the 1960s. Disease spread has been reduced through technological, organizational and biological advances such as vaccines, disinfection systems, hygiene routines, and combat zones for PD and ISA. A basis for increased biosecurity is to identify risk factors that can introduce infectious agents to a fish population and use the risk assessments to make – and follow – biosecurity plans (Lillehaug et al. 2015).

Transport of live animals is one of the most prominent risk factors for the spread of infectious diseases (Sommerset et al. 2021), and in aquaculture, wellboats are a significant route of infection (e.g. Murray et al., 2002). Nevertheless, there is still limited knowledge about infection in and from wellboats. Studies of the external hull and internal seawater systems have found multiple places problematic for access and cleaning (Cahill &Floerl 2019). Biosecurity on wellboats shall be safeguarded through e.g. technical standards, cleaning practices, and hygienic treatment of intake and discharge water from the vessels, but this is not done sufficiently today (Larsen et al 2020). It has previously been described that wellboats operate with small margins which can lead to shortcuts (Fenstad et al. 2008).

There has been a significant development with the use of recirculating aquaculture systems (RAS), which require new practices and knowledge about biosecurity and disinfection routines at hatcheries (Lazado &Good, 2021). Different facilities have different practices for production and disinfection, resulting in different microbiota composition at plants (Dahle et al., 2020; Dahle et al., 2021; Lazado &Good, 2021). In addition, there are major differences in mortality between hatchery facilities, which suggests an opportunity for improvement, e.g. through routines and technology (Tørud et al., 2019). Interview studies at RAS facilities, among others, have shown that employees consider some operational routines to be unwavering due to the plant’s design, but that many hatcheries still can improve their biosecurity routines (Tørud and Størkersen 2021). Larsen et al. (2020) urge for more knowledge so it can be possible to establish a best practice for RAS facilities.

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Results and conclusion

The aquaculture industry needs control of infectious agents and biosecurity. This document study shows how three Norwegian fishfarm and wellboat companies plan and control their procedures related to microflora, technology design and operational routines. The oral presentation will compare the companies' parameters and routines regarding for example filters, UV, washing and disinfection eliminate bacteria and viruses in RAS and wellboats.

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DECISION MECHANISMS IN NORWEGIAN AQUACULTURE: COMPARISON BETWEEN CONVENTIONAL AND NEW PRODUCTION SYSTEMS

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Introduction

The Norwegian salmon supply chain represents a global food system with a complex logistics network taking feed inputs from one part of the world and distributing products to different parts of the world after processing them in various locations. Salmon aquaculture is also heavily influenced by political decisions in terms of regulation of where farm sites and slaughter plants are located and legislation regarding feeds and medication use (Ziegler, et al. 2012). Although, production volumes have been increasing over the past decades, further growth is expected to diminish due to biological boundaries with only a 3% annual growth rate from 2019 to 2023 (Hoel and Howell 2021). Production using the conventional open-nets pens is limited under the current licensing regime and this is where new production systems can offer various advantages.

Sustainable growth of the aquaculture sector also needs to consider synergies between competing interests, the development of entire value chain and the corresponding infrastructure requirements. The current regulatory framework applies mainly to the conventional production systems, there is an urgent need to develop regulations that are aligned with the development of technical innovations in the aquaculture industry. A comprehensive understanding of the impact of regulations on decision making in the aquaculture supply chain is still lacking. COMPAREIT project is funded by the Research Council of Norway and addresses how new production systems should be regulated, administered, and where they could be best located, which are highly relevant questions for a sustainable development of the industry that safeguards other societal interests. This study focusses on mapping the key decisions in aquaculture production and the regulations that affect them.

Methods

The farming stage in the Norwegian aquaculture supply chain is particularly affected by regulations, volume constraints and is in need of technical development to increase production volumes in a sustainable manner (Ernst & Young 2021). This study investigates key decisions in the farming stage of the Norwegian aquaculture supply chain. The work focusses on all decision phases including strategic, tactical and operational planning for conventional and new production systems. Most key decisions are affected by regulations which are identified and compared for the conventional and new production systems. The work is based on a review of existing literature supplemented by interviews with aquaculture experts including researchers at SINTEF Ocean and industry partners in COMPAREIT project.

Results & Discussion

The Directorate of Fisheries, the County Authority, the County Governor, the Norwegian Coastal administration and the Norwegian Food Safety Authority are the key decision makers that govern the aquaculture sector by granting production licenses and regulating production locations and production volumes. The County Authority grants the aquaculture licenses and takes advice from Directorate of Fisheries, municipalities based on the area plan, the Norwegian Food Safety Authority based on the Animal Welfare Act and the Food Act, the County Governor based on the Pollution Act and Norwegian Water Resources and Energy Directorate based on the Water Resources Act if there are outtake of freshwater in case of land-based systems. Compared to conventional sea-based production systems, the regulations for new production systems are lacking.

To achieve its growth targets in a responsible manner, the Norwegian aquaculture sector must consider the multiple dimensions of sustainability in its regulations and decision-making processes by individual companies and not merely a branding exercise to gain consumer confidence. For instance, land-based production systems can potentially eliminate the negative impacts on wild salmon and reduce the overall impact of the salmon supply chain as production can take place close to major consumer markets in turn reducing emissions linked to transport to global markets, a significant portion of which takes place by air-freight (Thakur, Johansen, et al. 2020). On the other hand, land-based farming could have a negative impact on fish welfare because fish in land-based tanks is more densely packed compared to the 25.0 kg/m³ rule that applies for sea-based farming. The trade-offs between various dimensions of sustainability are, therefore, an important consideration in decision making and design of future regulatory frameworks.

Further work in COMPAREIT will focus on analysing the infrastructure requirements to achieve sustainable future production growth through implementation of new production systems using the input-output modelling approach. Future scenarios will be developed taking into consideration the current and future anticipated regulatory framework.

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START-UP AND PERFORMANCE OF A DENITRIFICATION BIOREACTOR IN A COMMERCIAL SMOLT PRODUCTION FACILITY (RECIRCULATING AQUACULTURE, AKVA-GROUP) IN MID NORWAY

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Introduction

To provide capacity for the increasing production volumes of Atlantic salmon smolt and post-smolt in Norway, land-based recirculating aquaculture systems undergo rapid technological development and upscaling. To meet economic and environmental expectations, fresh-water usage and emissions of NO_3-N and PO_4-P must be minimized. The zero-water concept (ZWC, AKVA Group, Norway) allows the removal of P by precipitation, and the removal of N by heterotrophic microbial denitrification. While the precipitation of P is an instant and easy to control chemical reaction, the stable control of the anoxic microbiological denitrification is more complex. To achieve a full performance of the facility at an early stage, protocols for rapid start-up and operational management for the denitrification must be followed. While literature describes start-up and operation of lab-based reactors, protocols for the start-up at commercial scale and (low) temperatures around ~13°C are lacking (Oboody, 2017). To assist RAS operators with these challenges, we provide a protocol that allows for the stable denitrification within 10 weeks in maturation.

The protocol was applied at a commercial smolt production facility in Mid Norway. This study reports the performance of the denitrification bioreactor during start-up under controlled conditions, as defined by AKVA group's standard operating procedure (SOP). Solutions for minimizing the risk for H_2S formation are given.

Materials and Methods

The RAS bioreactor is part of a smolt production facility in Mid-Norway. The system is characterized by the following key performance indicators: Max feed rate = 3.2t/d, operating temperature = 13-16°C, max salinity = 6ppt. The removal of solids is achieved by mechanical drum filters, the nitrification by fixed bed bioreactors with semi-automated cleaning technology (SAC, bio-media is circulated by water injectors), ozone is used to decrease turbidity, and degassing of ozone and CO_2 is achieved in a degassing tank.

The denitrification fixed-bed-bioreactor has the following measures: $l \times w \times h = 12.7 \times 2.2 \times 4.3\text{m} = 120\ \text{m}^3$ total volume. The relative volume of the fixed-bed bio-media account for 50 %, providing 60 m^3 of total bio-media volume. Multiplied with the specific surface area of the bio-media (800 m^2/m^3), a total surface area of 48,000 m^2 is available. The maximum flow rate is 100 m^3/h, accounting for a hydraulic retention time (HRT) of ~45 min and the treatment of ~35 % RAS-volume/d. Intake water for the denitrification bioreactor originates mainly from the fish tanks, and to smaller extend from rinsing water from the drum filters. The fixed-bed bio-media are connected to a semi automated cleaning system, consisting of 6 water injector pumps which facilitate continuous circulation of the bio-media. The start-up of the denitrification bioreactor was on 14th of Jan 2022. To create low oxygen concentrations, the HRT was was increased from 45 min to ~3h. To provide for chemical oxygen demand (COD), methanol was injected via an peristaltic pump to achieve a target ratio of NO_3-N:COD = 1:4.5 (stoichiometric optimum for complete denitrification with methanol). Given a background COD of ~100 mg/l, and a NO_3-N concentration of ~70 mg/l, 200 mg/l COD was supplemented. Water samples at the inflow and outflow of the reactor were analysed for dissolved oxygen, NH_4-N, NO_2-N, NO_3-N and COD twice daily. The first sampling took place during the first 10 days after start-up (14-20 Jan 2022), at high stocking densities (~180t total biomass) and feed input (~2.6 t/d), so elevated levels of nitrate are to be expected. A second sampling was taken approx. 1-month after start-up (08-11 Feb 2022). This sampling followed a vaccination event, with high water exchange and low nitrate levels to be expected. A 3rd sampling was taken 68 days after start-up (23 Mar 2022). This event followed a cleaning of the reactor 4 days earlier.

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Results

On day 1 after start-up, oxygen values in the outflow of the reactor remained well < 1mg/l. The average level of NO_3-N entering the reactor was ~67 mg/l. On day 1, about 10% of the NO_3-N entering the reactor was removed. The removal increased to 26% relative to the input on day 10 after start-up (Figure 1).

A 2nd sampling was carried out on day 27 after start-up. NO_3-N levels entering the reactor were ~32 mg/l and decreased to ~10 mg/l in the outflow, so by ~69% relative to the inflow values. A 3rd sampling was carried out on day 68 after start-up, NO_3-N levels entering the reactor were ~65 mg/l and decreased to ~10 mg/l, so by ~85% relative to the inflow values.
We expect that under decreased HRT (45 min/h), nitrate-N levels will be maintained below 75 mg/l, with substantially less water reuse.

Discussion and Conclusions

We found, that when HRT and COD are adjusted according to our recommended values, the denitrification increases rapidly after just a few days. According to project partners (R. Netzer, 2022), denitrifying microorganisms are detectable also inside the nitrification bioreactor. Therefore, facilitating optimum conditions for these bacteria inside the denitrification bioreactor will result in rapid colonization and reactor performance, hence conversion of NO_3 to N_2 gas. This was obvious in the increase of nitrate removal over the first 10 days of sampling.

The second sampling at low nitrate levels (day 27) revealed that this process is also strong at low levels of nitrate (~30 mg/l NO_3-N).

Almost complete denitrification was detected at day 68 post-start (3rd sampling). We therefore expect that at the 4th sampling in May 2022, when stocking densities, feed input and nitrate levels are high, and HRT at normal operational levels (45min/h), the performance of the reactor will allow maintaining nitrate levels below the maximum tolerable level of 75 mg/l NO_3-N.

The installation of an ORP-monitoring system (connected to SCADA) allows the precise adjustment of COD to achieve good denitrification, but also to decrease the risk of H_2S formation.

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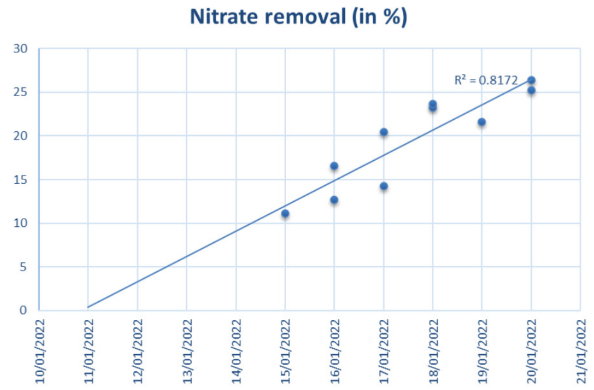

Figure 1: Decrease of nitrate levels by denitrification.

MULTI-USE SYSTEMS – A SOLUTION TO COMPETING INTERESTS IN OFFSHORE SPACE? FIRST LESSONS LEARNED FROM THE GERMAN NORTH SEA PILOT

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Introduction

The competition for marine space is a well-recognized challenge. The climate change commitments set by governments - triggering the development of renewable energy production-, the request for food and resource security - driving research for marine biomass production-, and a growing tourist sector next to societal challenges, increase the transformation of the marine realm. Multi-use offshore projects are experiencing a wide variety of challenges due to their different requirements for licensing, different needs in logistics and operational intensity. Particularly when extreme exceptional situations become a permanent factor excellent communication and intensive cooperation between the users and all those involved is essential. Experts are urgently required in all relevant areas, such as for the organisation of specified logistics, for specialized installation procedures, for complex legal contexts and pre-testing of materials and technologies for a successful functioning of the multi-use system.

Thus, the UNITED (Multi-**U**se offshore platforms demo**N**strators for boost**I**ng cost-effec**T**ive and **E**co-friendly pro**D**uction in sustainable marine activities) project investigates the technical, regulatory, economic, social and environmental viability of multi-use systems, which imply a combination of offshore wind farms with complimentary marine activities. Until today, there are only few successfully implemented multi-use systems, in spite of the encouragement of politicians and scientists. Hence, the project aim is to raise the technological, commercial readiness and innovation capacity of selected multi-use solutions, while reducing associated risks through the development of demonstration pilots in real life environment. This way, additional benefits to multiple sectors could be generated, infrastructural synergies could be created and the competitiveness of maritime businesses and economic opportunities could be increased within limited marine areas. In pursuing a more efficient use of ocean space and resources, synergetic effects shall be exploited, while reducing the overall environmental impact of a given use by combining it with another profitable activity.

This paper takes a special look at lessons learned from the German pilot. Experienced challenges and potential ways of solving unforeseen problems arising from the still largely unexplored multi-ise concept are presented.

Materials and Methods

In order to assess the benefits of offshore multi-use activities, five demonstration pilots, are set up in the North-, the Baltic- and the Mediterranean Sea, of which four pilots address the combination of offshore wind farms with aquaculture or tourism. The German pilot combines offshore wind energy research and aquaculture at a very exposed location (45 nautical miles from the shore), and thus reflects a situation where remote operational logistics are essential.

Major challenges had to be overcome due to the underdeveloped or missing framework conditions and unforeseen crises like COVID19 or a war in Europe. Problems arose because of restrictions affecting the supply and service industries. The pressure grew to focus on automation, alternative logistics, additional training and capacity building of personnel as well as health and safety issues.

The operational offshore phase of the project builds upon these developed strategies, which have only partly been tested so far. This phase of the project is ongoing and provids most valuable information on possible solutions in a still low developed area, as there are no practical examples from which experience can be gained. Limited experience of successful MUCLs under these new conditions requires research inputs to find new solutions to arising questions in support of the development:

- What reorientation of which industry sectors is necessary to implement the idea of offshore multi-use in practice?
- What innovative approaches are needed to integrate an additional user to an already established offshore project?
- What information must be available to potential partners for the successful implementation of new multi-use initiatives?

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Results

The planning of this project was done at least a year before the unexpected outbreak of COVID19. Within weeks after project approval these plans and some objectives were at risk and became redundant due to the outbreak. Underdeveloped or unavailable but existentially needed service for various areas from infrastructure to technical safety standards to legal and environmental expertise led to the development of diverse alternatives at the onset of the project. Several properly planned activities ready for timely implementation, had to be interrupted /postponed and subsequently to be cut short due to the need of implementing new structures. Alternatives had to be found to serve the original plans for data acquisition, particularly for monitoring instrument and their capabilities. Fortunately, the existence of a broad stakeholder register turned out to be most valuable to foster consensus building and gain improvement-oriented inputs. Thus, new adapted pilot solutions in one place mandatorily required a close communication not only within the pilot, but also between affected parties and this for the remainder of the project.

The pandemic demanded a new way of training and capacity building of personnel, creating teams with broad skills rather than single specialists. The operational phase teaches a lot of lessons learned to determine the future-oriented needs for a successful multi-use offshore operation. These results so far were not only used to educate internal personnel but also to facilitate a trans-disciplinary knowledge exchange of best practices from different fields, and to combine skills in order to be prepare the way for future operations.

Conclusion/Outlook

Risks and challenges of multi-use systems strongly vary between activities and sites, requiring adjusted planning and consideration of individual environmental, socio demographic and geographic conditions. However, when UNITED proves the success of multi-use activities that are ecologically, legally, socially and economically feasible in offshore wind farms, the way for future implementation of multi-use and co-location systems on a broader scale is paved. The need for time to exchange all available information and to cope with reservations and concerns should be considered in planning of future projects. The pandemic situation demanded an even broader and more flexible approach than ever anticipated. Documenting these lessons is strongly recommended as additional benefits to multiple sectors will be generated and diverse synergies are already created. If these will be implemented wisely, multi-use may support the EU Blue Growth strategy in mitigating the competition for marine space and promoting innovative economic uses and sustainable ecological processes.

This Project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no 862915.

NEONICOTINOIDS AND MARBLED CRAYFISH (*Procambarus virginalis*): CHRONIC AND ACUTE TOXICITY OF INSECTICIDE AND ITS ACTIVE SUBSTANCE

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Introduction

Neonicotinoids have gained a strong position in the insecticide market over the past thirty years. Their importance lies mainly in their effectiveness on various invertebrate organisms. In both insects and mammals, neonicotinoids act as agonists of nicotinic acetylcholine receptors (nAChRs). Due to the different structures of nAChRs, they show higher selectivity toward invertebrate receptors than vertebrates (Morrissey et al., 2015; Tomizawa and Casida, 2005). Due to the low toxicity of neonicotinoids to standard test species, they were not expected to significantly impact the aquatic ecosystem until later studies showed that aquatic invertebrates, especially insects, are much more sensitive to neonicotinoids (Morrissey et al., 2015; Sánchez-Bayo et al., 2016).

A suitable indicator species for toxicity tests is the marbled crayfish (*Procambarus virginalis*). Its breeding is undemanding; it has a fast life cycle, and, thanks to parthenogenic reproduction, it produces genetically identical offspring. Crayfish are suitable for laboratory experiments, given that they meet the 3R concept (Replacement, Reduction, Refinement). In crayfish, it is possible to observe changes in the organism at the molecular level due to the action of pollutants.

Material and methods

The pure insecticide acetamiprid (99 %) and the insecticide Mospilan 20SP, which contains 20.2 % of the active ingredients acetamiprid, were chosen to assess the effect of neonicotinoids on marbled crayfish. Adult marbled crayfish were used for acute and chronic toxicity tests.

The acute toxicity test on crayfish was performed according to OECD methodology no. 203 (OECD, 2013) with modifications for crayfish by Velíšek et al. (2013). Seven concentrations were used to test pure acetamiprid and eight to Mospilan 20SP. Each concentration was performed in duplicate. For acute toxicity tests, 180 individuals were used in total. Acute lethal toxicity of acetamiprid and Mospilan 20SP was assessed by the number of crayfish dying at individual test concentrations over a time period of 24 h, 48 h, 72 h, and 96 h median lethal concentrations (LC0, LC50, LC100). Lethal concentrations were determined by linear regression and probit analysis with a confidence interval of 95 % using the EKO-TOX program (v. 5.2, INGEO Liberec).

Based on the results of the acute toxicity tests, a chronic toxicity test was performed on crayfish. The crayfish were exposed to the effect of two different concentrations of the test substances (acetamiprid and Mospilan 20SP) dissolved in water for 20 days; after the exposure, the crayfish were left in pure water for another 10 days. All experimental groups were performed in two repetitions. A total of 160 crayfish were used. Tested concentrations were determined based on acute tests as 0.1 % and 1 % detected 96hLC50. Tissue samples for further analysis were taken for 10 and 20 days of exposure to the test substances and after 10 days of depuration. From each tested group, six individuals were always selected, from which a sample of haemolymph and tissues - hepatopancreas, muscle and gills - were taken after killing.

Biochemical analysis of proteins, lipid peroxidation (LPO), glutathione S-transferase (GST), reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) was performed on the tissue samples.

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Results

Acute toxicity (96hLC50) of acetamiprid was 0.8 mg.l^{-1} and of Mospilan 20SP 2.71 mg.l^{-1}. Based on acute toxicity tests was found the theoretical acute toxicity of Mospilan (96hLC50 = 4.0 mg.l^{-1}). According to the theoretical toxicity of Mospilan, especially at the beginning of exposure (48hLC50 = 34.7 mg.l^{-1}; 48hLC50 Mospilan = 10.73 mg.l^{-1}; 48hLC50 acetamiprid = 6.94 mg.l^{-1}), can be consider that additives contribute more significantly to the toxicity of Mospilan than the active substance acetamiprid alone.

Acetamiprid and also Mospilan affect levels and concentrations of oxidative stress and antioxidant biomarkers. Significant changes (p<0.1 and p<0.5) in tissues of marbled crayfish from acute toxicity tests were observed at concentrations of tested substances 0.5 mg.l^{-1}. In tissues from chronic exposure, significant changes were observed at concentrations corresponding to 0.1 % (acetamiprid) and 1 % (Mospilan) of 96hLC50.

Conclusion

The toxicity of neonicotinoids varies for different species. High differences are shown, especially between invertebrate and vertebrate organisms. The fact that standard test organisms are relatively resistant to their effects also contributed to their worldwide spread.

The marbled crayfish, as a representative of invertebrates, appears to be a suitable organism for toxicity testing. It is a relatively sensitive species. When using it in toxicity tests, evaluating several parameters, such as changes in behaviour and mortality, is possible. From tissue samples is possible to evaluate changes in the levels and concentrations of antioxidant biomarkers and oxidative stress biomarkers. It is also possible to evaluate biomarkers contained in haemolymph and histological changes in tissue samples such as muscles, gills or hepatopancreas.

Acknowledgement

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ANTIOXIDANT ENZYME ACTIVITY AND LIPID PEROXIDATION IN GILLS OF DISCARD SARDINE *Sardina pilchardus*

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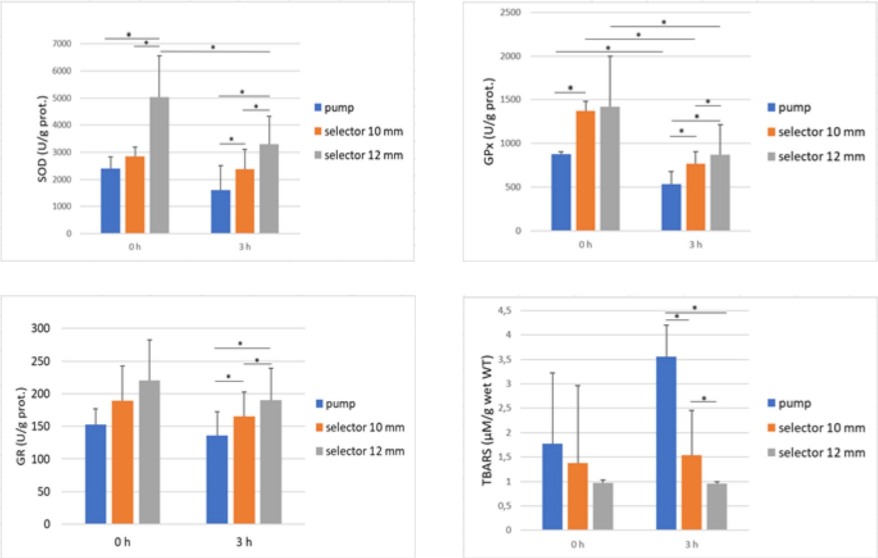
Introduction

In recent decades, the population of small pelagic fish such as sardine (*Sardina pilchardus*) has declined in the Mediterranean. In order to conserve fish stocks, various fishing techniques are being developed. One of them is the return of live, unwanted fish to the sea. However, the crowding in the purse seine, catching and handling onboard can expose fish to stress that affects their welfare, health and ability to survive after release (Marçalo et al., 2008; Waley et. al., 2021). Stress response of fish to commercial fisheries are poorly studied. Therefore, the objective of this study was to evaluate antioxidative defense and lipid peroxidation in sardine gills to detect early stress response during fisheries that use onboard pumping techniques in combination with fish selection to separate and return of unwanted fish to the sea.

Materials and Methods

A total of 160 live sardine (mean total length 138.20 ± 7.81 mm, mean weight 18.41 ± 3.43 g) were sampled during a commercial fishing by purse seine in the central Adriatic Sea. Fish was brought on board using the fish pump (Faivre, France) instead of handling them manually. Hauling time was around 90 to 115 min while the transfer period (by pump) lasted 10 to 30 min. After pumping fish were selected through a selector (selection grid 10 mm and 12 mm) and placed into 400 L tanks (70 fish/tank). Fish for analysis were collected immediately and 3 h after pumping and selection procedures. Gill samples of each fish were homogenized in phosphate buffer (pH 7.4, PBS, Sigma), and centrifuged at 9,000x *g*/4^{o}C/10 min. The antioxidant enzyme activities and lipid peroxidation were measured from supernatants. The activity of superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) was determined by Randox commercial kit (Ireland) and analyzed on the biochemical analyzer ARCHITECT c4000 (Abbott, USA). Lipid peroxidation, expressed as thiobarbituric acid reactive substance (TBARS) was measured in accordance with Babić et al. (2016) using the plate reader (Infinite M200, Tecan). Statistical analyses were performed using SigmaStat and SigmaPlot Statistical software version 11.0 (Jandel Corp., San Rafael, USA). A $p < 0.05$ was regarded as statistically significant.

Figure 1. Antioxidant enzyme activities and lipid peroxidation levels in sardine gill tissue immediately (0 h) and 3 h after pumping on board and pumping combined with selection through grid 10 mm and 12 mm. Values are means ± S.D. The asterisk denotes a significant difference between groups ($p < 0.05$).



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Result and Discussion

The effects of fishing operations on antioxidant enzyme activities and lipid peroxidation are presented in Figure 1.

Significantly higher SOD and GPx activities were recorded in fish sampled immediately after pumping combined with selection through the grid of 12 mm than in other groups. Higher but not significant GR value was also recorded in the same group. Three hours after fishing, decreasing SOD level was significant only in fish pumped and selected through 12 mm grid while GPx activity significantly dropped in all groups. GR activity was also decreased but not significantly. Opposite to antioxidant enzyme, TBARS levels were lower immediately after capture than 3 hours later in all groups. However, significant increase was noted only in fish subjected to pumping procedure only. The obtained results demonstrate that fish transferred from purse-seine onboard and returned to the sea by pump induce less damage to the fish as they carry a mixture of fish and seawater that protects the fish from shocks. Additional selection through selector with different grid size could help to release unwanted sardine to the sea but induces higher level of stress. However, decrease in enzymatic activity three hours after capture indicates that the fish gills could recover after returning to the sea.

Conclusion

Fish welfare is affected by commercial fishing. Improvements in commercial fishing practices could increase chances of survival of fish that are returned to sea.

Acknowledgements: The research is co-financed by the EU - European Maritime and Fisheries Fund (EMFF).

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THE MICROBIOTA KNOWS: HANDLING-STRESS AND DIET TRANSFORM THE MICROBIAL LANDSCAPE IN THE GUT CONTENT OF RAINBOW TROUT IN RAS

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Background
The aim of the present study was to characterize the effects of handling stress on the microbiome in the intestinal gut contents of rainbow trout (*Oncorhynchus mykiss*) from two different breeding lines fed with a plant-based diet. Two experimental diets were provided for 59 days to all female trout and half of the fish were shooed with a fishing net twice per day to induce handling-stress (groups 0 and 1) in a fully recirculating aquaculture system (RAS). Experimental diets were formulated according to commercial trout diets differing in fish meal (35 % diet F, 7 % diet V) and plant-based proteins (47% diet F, 73% diet V).

Results
No differences in performance parameters were found between treatment groups. By using 16S rRNA amplicon sequencing of the hypervariable region V3/V4 we examined the microbial community in the total gut content of fish at the end of the trial. We discovered no significant differences in alpha diversity induced by one of the two factors within both trout lines. However, the microbial composition is significantly driven by an interaction of stress and diet in gut contents of trout line A. Otherwise, in trout line B the main factor affected the microbial composition was stress. The communities of both breeding lines were predominantly colonized with bacteria from the phyla *Fusobacteriota, Firmicutes*, *Proteobacteria*, *Actinobacteriota* and *Bacteroidota*. The most varying and most abundant taxa have been found to be *Firmicutes* and *Fusobacteriota*, where at the genus level *Cetobacterium* and *Mycoplasma* are key components in terms of adaptation.

Conclusion
We conclude, that microbial gut composition is highly influenced by stress which is presupposed by diet formulation and trout origin but constant microbial diversity and performance parameter.

APPETITE FOR PRODUCTION – FEEDING AND DIGESTION STUDIES IN THE EMERGING SEA CUCUMBER SPECIES *Parastichopus tremulus*

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Introduction

The red sea cucumber, *Parastichopus tremulus*, is an emerging aquaculture candidate species which in recent years has drawn attention from fishers, farmers, and traders (Landes et al. 2019; Christophersen et al. 2021, 2022; Schagerström et al. 2022). The species may be suited for cultivation all along the Norwegian coast, as its geographical distribution in the north-east Atlantic is from the Barents Sea in the north to the Canary Islands in the south. Deposit feeding species such as *P. tremulus* are able to ingest and utilise nutrients from particulate organic matter and have therefore been suggested for inclusion in integrated multi-trophic aquaculture (IMTA) systems. It has been suggested that particular waste (sludge) from fish farms could be suited as feed or a feed ingredient for sea cucumber aquaculture. The Norwegian salmon aquaculture production annually results in an estimated 500 000 tonnes of sludge (Aas 2021). This nutrient-rich side stream consists of uneaten feed and faeces, of which only a minor fraction currently is collected and used for low-value applications. There is an increased interest in valorising this waste stream. Performance of *P. tremulus* in aquaculture is unknown and species specific biological knowledge gaps need to be filled for a potential industry to succeed. In particular, little information exists on its nutritional requirements and efficiency in extracting organic matter from different feed sources (Hauksson 1979). The aim of this study was to assess the feasibility of formulating sea cucumber feeds from salmon aquaculture sludge.

Methods

This study was part of the research project "Emerging species for sea cucumber aquaculture", investigating the aquaculture potential of several tropical and temperate species from Norway and South Africa. The individuals used in the experiments were obtained from the fjord system around **Ålesund** in Møre and Romsdal county in the north-western part of Norway (62°N-6°E). Before the trials, the animals were acclimated in flow-through tanks supplied with unfiltered seawater from 40 m depth. For the feeding trial, nine sea cucumbers (mean weight 98.7 ± 76 g) were held individually in flow-through aquaria (25 L) supplied with filtrated seawater (1 **µ**m). Three different experimental feed mixes were given in a rotating experimental design. Sea cucumbers in three aquaria (n=3) where given the same feed for one week, and at the end all individuals had been presented with all feeds. The experimental feeds were composed of sand (0-1 mm) mixed with different ratios of either dried *Sargassum thunbergii* powder, a common ingredient in feed for Asian sea cucumber species, or dried aquaculture sludge acquired from a land-based Atlantic salmon smolt facility. Two experimental feeds were composed with sludge (50 and 75 % (v:v)) and dried *S. thunbergii* (50% v:v) was used as control. The sludge raw material contained 27.9 % protein, 4.4% lipids, 33.2% carbohydrates and 22.2 % ash with an energy content of 953 kJ/kg. The *S.thunbergii* powder was lower in energy (551 kJ/kg), protein (10.2%) and lipids (0.4%) but higher in carbohydrates (39.7%) and ash (41.8%). Both sludge feeds were added 1% guar gum as a binder to prevent leaching of nutrients to the water. All feeds were supplied in excess (30% dry weight/wet weight animal/week) and faeces collected daily. After 7 days of collecting faeces, remaining feed was removed from the tank and animals starved for 72 h before the entire sequence was repeated with a new feed. Feed and faeces samples were analysed for total organic matter (TOM) by drying and ash weight determination. These data were used to calculate daily ingestion rates (DM day^{-1}) and organic matter extraction efficiency (TOM %).

Results

The TOM content of the feeds were 13.4 ± 0.5 % (control), 24.6 ± **0.3** % (50% sludge) and 44.1 ± 0.5 % (75% sludge). Feed intake (% DM feed × WW animal $^{-1}$ day^{-1}) was significantly higher for the control feed (0.85 ± 0.62 %) than for either of the experimental feeds (0.02 ± **0.01 % and** 0.04 ± **0.04** %, respectively). On average, digestibility was estimated to 32.5 % (control feed), 29.6 % (50% sludge) and 57.8 % (75% sludge), and large individual and day-to-day variation was seen.

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Discussion/Conclusion

In a circular economy perspective, the utilisation of aquaculture sludge as feed for sea cucumbers is an attractive concept. Our results indicate that digestibility of sludge in *P. tremulus* is high, suggesting that it could have a potential application as an ingredient in feeds for sea cucumber aquaculture. However, additional research is needed on its long-term effects on growth and health parameters in *P.tremulus*.

Unfortunately, since regulatory restrictions currently prohibit the use of aquaculture sludge as feed for animals intended for human consumption, legislation needs to change to make this concept feasible in the future. Caution should also be taken regarding potential accumulation of toxic and undesirable substances in the sludge. A step-by-step approach to understand the requirements of the *P. tremulus* in captivity is ongoing, but much more knowledge is needed to establish reliable procedures for aquaculture production. Biological and technological constraints still hinder the development of large-scale aquaculture production of *P. tremulus*, and substantial research is needed on the areas of reproduction, nutritional requirements, and water quality.

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TOWARDS HUMANE SLAUGHTERING OF SNOW CRAB (*Chionoecetes opilio*)

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Introduction

The snow crab fishery takes place in the Barents Sea far from land and therefore majority of the crabs are slaughtered on board the fishing vessels. A common slaughter method is the use of a splitting machine. This machine works by fixating the crab's walking legs and claws and flipping off the carapace before splitting the crab in two using either a knife blade or a water jet. Despite the widespread use of this method, there is little evidence as to whether this renders the crab unconscious, or if there could be alternative methods that may be more humane. In our study we aimed to investigate three different slaughter techniques (chemical, mechanical and electrical) and assess the welfare using behavioural and neurological (EEG of thoracic ganglion) measurements to examine response and loss of consciousness and death.

Materials and methods

Three slaughtering techniques were tested; chemical, mechanical and electric. The chemical method was injecting saturated potassium chloride (KCl) directly into the heart using a syringe. Welfare was evaluated by scoring responsiveness of eyes, antenna, mandibula, chelae, walking legs, claw and tail along with EEG. The mechanical method was splitting the crab in two using a large kitchen knife. Welfare was evaluated as above. The electric stunning was carried out using a dry stunner 50 Hz, 220 V AC. The crab was placed on a metal plate and the electrodes were placed either on the carapace or fixated on the legs to simulate the splitting machine onboard fishing vessels.

Results

Of the three methods, correct splitting gave what appeared to be a quick epileptic seizure followed isoelectric line indicating a cessation of nerve signa i.e. death. However, if the splitting was only slightly off the middle, signal would continue, even when the crab was non responsive during the behaviour tests. KCl had varying results from fast cessation of signal to continuing signal until euthanasia. Eyes, antenna, mandibula, chelae, walking legs, claw and tail were all negative on the responsiveness test, but EEG could still sometimes show signals during the test. Electrical stunning also gave epileptic seizure, but some individuals appeared to regain a "normal signal" after 3-4 minutes which coincided with increased response to stimulation, whereas other did not regain either normal signals or responsiveness. With electric stunning there was in general good correlation between behavioural response during stimulation and increased amplitude and frequencies of signal measured by EEG.

Conclusion

The preliminary data indicates that here is not always a good correlation between behavioural responses and neural activity (indication of potential consciousness) during slaughter of snow crab when using splitting or injection of KCl. Of the three methods, splitting and electrical stunning appears to be the most promising. We propose a combination of the two, - a stun splitter, to be a potential new and humane method for slaughtering snow crabs.

INVESTIGATION OF LIFE-HISTORY TRAITS FOR *Ophryotrocha craigsmithi*: IMPLICATIONS FOR ITS ROLE IN INTEGRATED MULTITROPHIC AQUCUALTURE SYSTEMS

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Introduction

High Densities of the deep-sea polychaetes belonging to the genus Ophryotrocha sp. have repeatedly been observed to colonize and feed on aquaculture waste accumulating under salmonoid fish cages in both Norway (Hansen et al., 2011, Eikje, 2013, Brennan, 2018) and Canada (Salvo et al., 2015, Armstrong et al., 2020).

There is an increasing interest in integrating these polychaetes in circular production systems for the mitigation of organic fish-waste and for the future production of a new aquaculture feed ingredient (Nederlof et al., 2019, 2020). Information regarding life-history traits are essential for the successful integration of these polychaetes and has until now been undescribed for one of the main candidate species in Norwegian waters, *Ophryotrocha craigsmithi*. Information about growth, fecundity and larval development provide elements necessary for more refined estimates of bioremediation potentials performed by populations of polychaetes colonising benthos under fish farms. In addition would knowledge about population turnover for *O. craigsmithi* be important for future harvesting strategies. Here we present life history traits for the polychaete species *O. craigsmithi*.

Materials and methods

Collection

Polychaetes were collected under an aquaculture farm at approximately 200 meters depth outside the west coast of Bergen, Norway. For this equipment developed to both attract and facilitate the establishment of epibenthic polychaetes where used. *O. craigsmithi* was then successfully reared in the laboratory facility for approximately five months.

Growth

Polychaetes were held in flow-through tanks with temperature and salinity (~ 8-9 °C and 34-35 ppm) similar to their natural habitat. Holding tanks were sampled for individuals used in a growth experiment with the aim to determine growth throughout the life cycle of *O. craigsmithi*. For this 3x3 groups of cohorts representing small, medium and large sized individuals were divided in to separate 8,5 L flumes and fed ad libitum with fish pellets. Feed was provided in excess to resemble fish farm conditions, where food is not assumed to be a limiting factor. Chambers were photographed on a weekly basis and images were later analysed in order to extract survival and length data of polychaetes. Photographing chambers allowed gentle handling of polychaetes and reduced stress to a minimum.

Fecundity

The total fecundity of *O. craigsmithi* was measured through the process of induced spawning by increasing water temperature in known volumes of water. Individuals of different sizes were collected from the laboratory holding tanks and eggs were later counted and measured with the help of a PAMAS field laser counter.

In this study the total fecundity was defined as the amount of eggs counted for one individual of a given size, including both mature and immature eggs. The size of mature eggs were defined in a separate experiment were groups of *O. craigsmithi* were allowed to spontaneously spawn and eggs were collected, investigated under a microscope and later measured with a PAMAS laser counter.

Histology

Individuals ranging in size from ~ 4-14 mm were selected for histological sectioning in order to investigate the presence of male/female gametes and for the indication of size for the onset of Oocyte production in females. Polychaetes were relaxed in isotonic magnesium chloride, measured under a dissecting microscope and later fixed in Davidson`s fix before casted in to paraffin and longitudinally sectioned in 3 μm thick sections and analysed.

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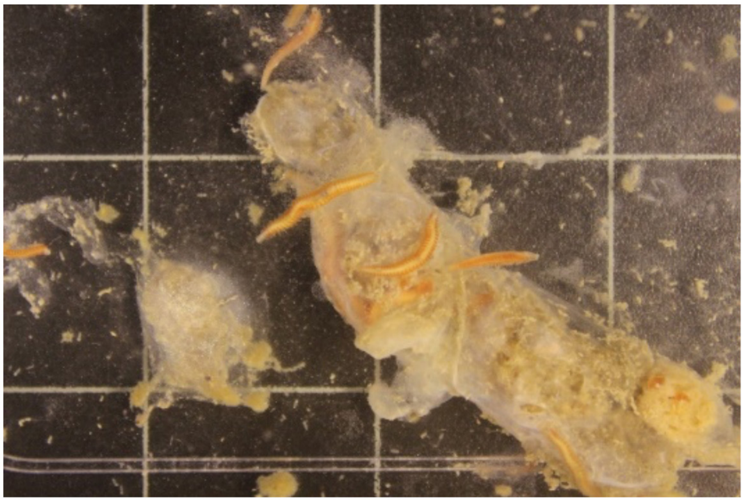

Figure 1) Individuals of O. craigsmithi occupying growth flumes. O. craigsmithi crawling inside and around a mucus construction containing organic material and bacteria. White squares 30x30 mm.

Results

Preliminary results show that it takes approximately two weeks for the small group of *O. craigsmithi* (3-5 mm long) to double in size. The maximum size of adult individuals was reached around 20 mm. Fecundity measurements indicate that mature adult females can contain around 1000 eggs within the size range 90 to 190 μm. A mix between mature an immature eggs was almost exclusively observed in al spawning size-groups of *O. craigsmithi*, this was also supported by histological sections. Fecundity and histological measurements together indicate that females can start egg production with few, but mature eggs from about 6 mm in length. Histological sections has not yet revealed any individuals producing both male and female gametes, pointing at separate sexes for this species. Additional observations made for *O. craigsmithi* in the laboratory study was that they possess a free swimming larvae stage with the first segment appearing after a few days. *O. craigsmithi* was also observed to efficiently collect food and bacteria in 3D mucus complexes (Figure 1). Results from this study increases the understanding and biological requirements for a successful incorporation of *O. craigsmithi* in systems like integrated multitrophic aquaculture.

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DIETARY CARBOHYDRATE SOURCES FOR EUROPEAN SEABASS REARED IN RECIRCULATING SYSTEMS: IMPACTS ON DIGESTIBILITY AND WASTE PRODUCTION

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Introduction

Recent substitution of fish meal with alternative ingredients has led to the inclusion of more carbohydrates in fish feeds (Sinha et al., 2011). Several studies have addressed the effect of novel raw materials on growth and overall fish performance (Francis et al., 2001) but little is yet known about the impact of those fibrous ingredients on aquaculture system performance. Nonetheless, waste production and managament becomes more and more relevant as recirculating aquaculture keeps growing. In view of this, fish farming in closed systems poses more challenges when faecal volume gets higher due to lower diet digestibility, and faecal waste is prone to disintegration due to poor quality (Kokou & Fountoulaki, 2018). Therefore, the present study examined the impact of different carbohydrate sources on nutrient digestibity and subsequent faecal excretion, with the aim to identify dietary ingredients which improve waste removal efficiency.

Materials and methods

A trial was conducted in which juvenile European seabass (*Dicentrarchus labrax*) was fed restrictively (1.7% of body weight) with eight experimental diets in triplicate; seven diets included a different carbohydrate source in the form of non-starch polysaccharides, chitin and keratin, while one served as control (basal diet). The test diets consisted of 85% basal mixture, diluted with 15% of each test ingredient. The ingredients examined in the current setup were: 1) wheat dried distillers grain with solubles; DDGS, 2) shrimp-shell meal; SSM, 3) type I hydrolyzed feather meal; FM-I, 4) type II hydrolyzed feather meal; FM-II, 5) insect meal; IM, 6) single-cell protein; SCP, and 7) seaweed protein; SWP. To evaluate the impact of diet on nutrient digestibility and waste production, each tank was equipped with an individual swirl separator for faeces collection. Nutrient digestibility, calculated as apparent digestibility coefficient (ADC), was measured after 3 weeks of acclimatization to the respective diet, based on yttrium oxide (0.2 g/kg feed) as an inert marker. Additionally, faecal waste production was estimated via organic matter (OM) digestibility values, whilst faecal recovery was determined by quantification of the OM collected via swirl separators over a 48-hour period.

Results & Discussion

The present study demonstrated that European seabass is able to digest carbohydrate-rich diets, at least up to 15% inclusion levels, despite being a typical carnivorous species. However, the ADC of all nutrients but fat differed among dietary ingredients, with the lowest values for ADC crude protein and carbohydrate being reported for SWP and DDGS, respectively. Similarly, OM digestibility was higher for the ingredients of animal origin compared to the plant-based ones, which can be likely attributed to the high presence of indigestible non-starch polysaccharides (e.g. lignin, cellulose, pectins) in the latter, as previously suggested for European seabass (Fountoulaki et al., 2022). This consequently denotes that faeces production is lower for the animal-based diets and thus a smaller volume of faecal waste needs to be treated in a recirculating aquaculture system (RAS) under these conditions. In addition, SSM resulted in the highest faecal recovery, followed by IM (Table 1). This suggests that chitin is possibly a polysaccharide with binder capacity which improves faecal stability and facilitates faecal removal via basic mechanical filtration methods. This is in line with results previously reported for other insoluble fibres (Amirkolaie et al., 2005). As such, chitinous feeds are exprected to accommodate RAS performance since they may reduce the accumulation of small particles in the recirculation loop. Overall, the obtained results show that the type of dietary carbohydrate determines faecal quantity and quality with potential implications on system performance and hence therof inclusion should be considered during formulation of RAS aquafeeds.

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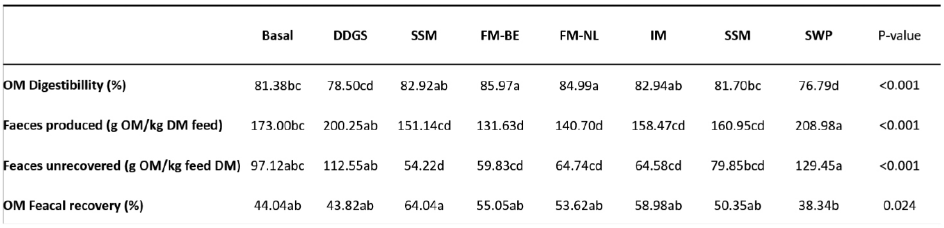
Table 1. Parameters related to faecal quantity and quality as determined for the different ingredients tested in the present study.



	Basal	DDGS	SSM	FM-BE	FM-NL	IM	SSM	SWP	P-value
OM Digestibillity (%)	81.38bc	78.50cd	82.92ab	85.97a	84.99a	82.94ab	81.70bc	76.79d	<0.001
Faeces produced (g OM/kg DM feed)	173.00bc	200.25ab	151.14cd	131.63d	140.70d	158.47cd	160.95cd	208.98a	<0.001
Feaces unrecovered (g OM/kg feed DM)	97.12abc	112.55ab	54.22d	59.83cd	64.74cd	64.58cd	79.85bcd	129.45a	<0.001
OM Feacal recovery (%)	44.04ab	43.82ab	64.04a	55.05ab	53.62ab	58.98ab	50.35ab	38.34b	0.024

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ENVIRONMENTAL DRIVERS OF FISH MORTALITY ALONG AN INSHORE-OFFSHORE GRADIENT IN SCOTLAND

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Introduction

Aquaculture is the fastest growing food production sector and currently accounts for the majority of human fish consumption (FAO 2020). In Scotland, the salmon aquaculture industry consists primarily of sea cages within fjordic sea lochs which provide an environment that is relatively sheltered from wave exposure. However, the restricted water exchange may create several problems, including reduced temperature and dissolved oxygen gradients, increased mortality from harmful algal blooms, and increased risk of ectoparasites and disease transmission. Recently, interest has increased in the possibility of moving salmon aquaculture 'offshore' into more exposed environments with higher energy currents and waves (Morro et al. 2021). While the more dispersive environment may reduce effects of waste and decrease exposure to parasites such as sea lice, expectations for the effect on fish health and wellbeing are largely speculative, and the interactions of the multiple stressors faced by fish in offshore farms may result in both synergistic and antagonistic effects (Matzelle et al. 2015; Orr et al. 2020). Here, we use health and welfare data from sea cage farms in western Scotland representing a gradient of wave exposure as an offshore proxy to assess the effects and interactions of environmental variables and management treatments on fish health and mortality using hierarchical Bayesian models.

Materials and methods

Eight sea cage farms in western Scotland were selected based on the similar characteristics of the Atlantic salmon (*Salmo salar*) smolts used for stocking and their contrasting locations and physical conditions (Fig. 1). Data were collected at each farm from July 2018 – March 2020 by a collaborating fish farming company, which included mortalities, number of sea lice per fish (*Caligus sp*., *Lepeophtheirus salmonis*), Amoebic Gill Disease (AGD) score (1-4), and administration of anti-lice (Thermolicer, Hydrolicer, SLICE, Salmosan, Alphamax) and anti-AGD treatments (Freshwater, H_2O_2).

Wave exposure at each site was characterised using the 50-year significant wave height as predicted by the West Scotland Wave Model (Hardwick 2020). Environmental variables were extracted from the WeStCOMS hydrodynamic model, which predicts hourly conditions at 10 depths based on observed environmental forcings (Aleynik et al. 2016, Dale et al. 2020). The daily 90th quantile at each farm was calculated for temperature and water velocity at 6 m depth, as well as wind velocity.

The joint effects of environmental and health variables on daily mortality rate were estimated using a hierarchical Bayesian model. The full model used logit-transformed mortality rate as the response, predicted by effects of wave height, temperature, wind velocity, water velocity, fish abundance, number of adult lice, average AGD score, days since mechanical or chemical lice treatments, and days since anti-AGD treatments. We included interactions between wave height and each variable, explored lagged impacts, and incorporated effects of fish cages nested within each site. We used regularlised horseshoe prior distributions for each slope.

Analyses were performed in R 4.1.3 with the package *brms* v. 2.16.3 (Bürkner 2017), which fits hierarchical Bayesian models using Markov Chain Monte Carlo with the statistical platform Stan (Stan Dev. Team, 2022). For each model, we ran four chains of 3,000 iterations each, with the first 1,500 as warmup. Suitability for interpretation was assessed with posterior predictive checks, ensuring that all < 1.1, and confirmation that no divergences occurred during the sampling.

Results

The model development is currently in progress and the abstract will be updated to include the results.

Preliminary analyses show suggestive correlations ($|r| > 0.2$) between mortality rate and temperature, lice density, and time since chemical lice treatments (Fig. 2). Further, when sites are classified as 'inshore' or 'offshore', fish at offshore sites tend to be smaller and thinner ($P < 0.05$), while inshore fish show higher levels of cortisol and reduced density of adult sea lice in the summer ($P < 0.05$).

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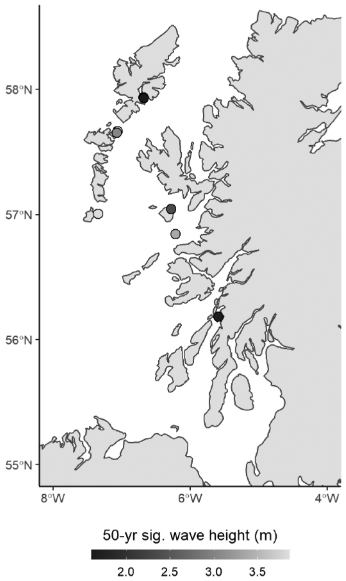

Figure 1. Study farms in Scotland.

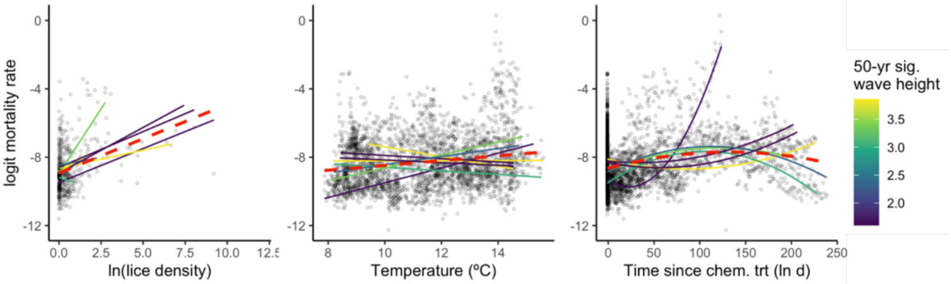

Figure 2. Univariate patterns with mortality (solid: farm-level; dashed red: global).

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MANILA CLAM AND MEDITERRANEAN MUSSEL AQUACULTURE IS SUSTAINABLE AND ACT AS NET CARBON SINK

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Introduction

Aquaculture is a globally expanding industry that contributes to feeding an increasing global population. Shellfish cultivation is one of the largest sectors of aquaculture and one of the few food productions that have the potential capacity of acting as carbon sink. In fact, more than 90% of bivalve shells are calcium carbonate ($CaCO_3$), synthetized during biocalcification process, which incorporates a molecule of CO_2. Manila clam (*Venerupis philippinarum,* Adams&Reeves, 1850) and Mediterranean mussel (*Mytilus galloprovincialis*, Lamarck, 1819) are two of the major groups of cultivated shellfish. Our aim was to assess the potential role of those two bivalve species in the overall marine carbon balance using an ecosystem approach, and to evaluate if they can be definitely regarded as carbon sink.

Materials and Methods

The contribution to CO_2 emissions (as CO_2 eq./kg of fresh products) due to mollusk farming has been also calculated as carbon-source term by means of Life Cycle Assessment (LCA). LCA is nowadays the most shared and accepted tool for evaluating the environmental impacts of aquaculture productions. The carbon sequestration capacity during biocalcification process has been also calculated. As a case study, the Sacca di Goro coastal lagoon (Northern Adriatic Sea, Italy) has been considered, because it is the premier site in Europe for clam farming, and one of the most important for mussels.

Results

Our study has shown that for each kilogram of harvested and packaged clams and mussels, shell formation throughout the mollusk growth allows to permanently capture 254 and 146 g of CO_2, in the face of 22 and 55 g CO_2 eq. emitted for farming, respectively. As a result, clams and mussel aquaculture could be considered as a carbon sink, with a net carbon capture capacity of 233 and 91 g CO_2/kg of fresh product, respectively. In a wider context, bivalve aquaculture could be included in the carbon trading system and played a role towards the carbon-neutral economy.

GROWTH PERFORMANCE, AMINO ACID AND FATTY ACID COMPOSITION OF GILTHEAD SEABREAM (*Sparus aurata*) FED ALGAE MEAL DIETS

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Introduction

Aquaculture sector is thriving as a response to the rapidly increased demand for seafood products. The sector is using, 18 million tons of wild fish that are targeted for fishmeals and fish oils in aquafeeds (FAO 2020), thus affecting the sustainability of marine ecosystem. This has led the research to focus on environmentally sustainable sources of proteins and lipids as replacements of fishmeal and fish oil. Microalgae biomasses seem to be potential feed ingredients containing sustainable amounts of essential amino acids, essential fatty acids, vitamins, and pigments, that could be produced with a low environmental footprint (Nagappan et al. 2021). This study aims to investigate the effect of dietary inclusion of algae meal on the growth performance, amino acid composition and fatty acid composition of the white muscle of gilthead sea bream (*Sparus aurata*).

Material and methods

The experimental trial was conducted at the Department of Ichthyology and Aquatic Environment, University of Thessaly, in Volos, Greece. Briefly, 360 individuals *S. aurata* (initial mean weight 6.43±0.04g) were distributed randomly to six 250l tanks. Two experimental diets were formulated to be isonitrogenous, isolipidic, and isoenergetic with diet 1 (PA) consisted of 8% *Phaeodactylum tricornutum*, and diet 2 (HA) consisted of 8.23% *Schizochytrium limacinum*. Each diet was assigned to triplicate groups of 60 fish per group. The trial lasted 45 days, after 15 days of acclimatization. The fish were fed three times per day *ad libidum*. Fish were weighted individually at the beginning and end of the experimental trial under anaesthesia. Feed consumption was recorded daily in order to evaluate accurately values for feed utilization.

Results

The results showed that weight gain, feed consumption, specific growth rate and survival did not have statistically significant differences ($p>0.05$) between fish fed the diet consisted of *Phaeodactylum tricornutum* (PA) and the diet of *Schizochytrium limacinum* (HA). Fish fed with PA had statistically significant ($p<0.05$) lower feed conversion ratio and higher protein efficiency ratio (Table 1).

Amino acid composition revealed a strong positive correlation, highly significant ($p<0.05$) between diets and the white muscle of fish in each treatment. Moreover, fatty acids analysis showed a significant decrease ($p<0.05$) of eicosapentaenoic acid (EPA) and an increase ($p<0.05$) of docosahexaenoic acid (DHA) in white muscle of seabream fed the HA diet, compared to the PA treatment. Arachidonic acid (ARA) of white muscle was unaffected ($p>0.05$) between the dietary treatments.

Table 1: Growth performance parameters of gilthead seabream fed the experimental diets.

	PA	HA
Final Weight (g)	26.54 ± 0.34^a	26.62 ± 0.35^a
Weight gain (g)	20.07 ± 0.34^a	20.22 ± 0.35^a
Feed consumption (g/fish)	15.48 ± 0.53^a	17.89 ± 0.78^a
Specific growth rate (SGR, %/day)	3.10 ± 0.02^a	3.13 ± 0.02^a
Feed conversion ratio (FCR)	0.81 ± 0.01^a	0.93 ± 0.01^b
Protein efficiency ratio (PER)	2.92 ± 0.04^b	2.52 ± 0.04^a
Survival (%)	98.88 ± 0.55^a	99.44 ± 0.55^a

Values are presented as means±standard error. Means sharing the same superscript are not significantly different from each other ($P<0.05$).

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Discussion

The results of this study showed that the inclusion of algae meal in *S. aurata* diets did not affect growth performance and amino acid deposition. The lower FCR and higher PER exhibited in fish fed the PA diet denotes a better protein utilization of *P. tricornutum* diet than *S. limacinum* by *S. aurata*. In terms of aquaculture sustainability, the substitution of fish oil with heterotrophically produced microalgae in salmon feeds proved the nutritional feasibility of low trophic level organisms such as algae in aquaculture fish feed (Kousoulaki et al., 2017). In accordance with the results of this work, fishmeal replacement with the microalgae *P. tricornutum* in started diets of *S. aurata* showed that SGR was not affected (p>0.05) and there was an increase of saturated fatty acids in fish (Atalah et al. 2007). Moreover, the inclusion of 2.5% *P. tricornutum* in finishing diets of gilthead sea bream did not significantly change growth, feed utilization parameters and muscle fatty acid profile (p>0.05) (Ribeiro et al. 2017). Furthermore, the 2.5% inclusion of *Schizochytrium* sp. as lipid source and especially DHA source in microdiets of *S. aurata* had no negative effect on larvae (Ganuza et al. 2008). In contrast to the forementioned authors, Eryalçin & Yildiz (2015) reported that the replacement of fishoil with dried *Schizochytrium* sp. meal had a negative effect on growth of seabream although the DHA was increased.

The differences found in the fatty acid profiles on the muscle tissue of fish are characteristic to the fatty acid's profiles of each algae species as *S. limacinum* is known for being richer in DHA and poorer in EPA compared to the *P. tricornutum* and *S. limacinum* has been used successfully in the diets of seabream and other species, mainly for fishoil replacement. According to Santigosa et al. (2021), the growth performance of seabream fed 3.5% *Schizochytrium* sp. oil as replacement of fishoil was unaffected and fillet fatty acid composition was in accordance with the diet fatty acid composition, suggesting that dietary microalgae could lead to an environmentally friendly final product rich in n-3, n-6 fatty acids. The dietary inclusion of algae meal (*Phaeodactylum tricornutum* and *Schizochytrium limacinum*) does not influence growth performance but there is an effect on FCR, PER, amino acid deposition and fatty acid composition of the white muscle of seabream (*Sparus aurata*) follows the fatty acid and amino acid composition of the two algae suggesting they can be used and produce a final product rich in n-3 and n-6 fatty acids. Algae meal biomass is promising ingredient to move the formulation of sea bream feed to a more sustainable future.

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NUTRITIONAL EVALUATION OF ULTRA-HIGH PROTEIN AND UNLTRA-HIGH PROTEIN LOW-OLIGOSACCHARIDE SOYBEAN MEALS IN DIETS OF POST-SMOLT ATLANTIC SALMON (*Salmo salar*)

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Introduction

Soy is one of the most common plant-based protein ingredients in aquafeeds because of its consistent nutrient density, favorable amino acid profile, good palatability and high availability. However, the main factor limiting the extended use of conventional solvent extracted soybean meal (SBM) in salmon diets is the presence of anti-nutritional factors like oligosaccharides, raffinose, saponins, etc., which have been shown to reduce digestibility, growth, and induce enteritis in the distal intestine of Atlantic salmon. To overcome this issue, new varieties of SBM called ultra-high protein (HP, 60.9% crude protein) and ultra-high protein low-oligosaccharides (HPLO, 60.5% crude protein) have been developed. The objectives of this study were to 1) determine nutrient digestibility of HP and HPLO, 2) evaluate the effects of dietary inclusion of HP and HPLO on growth performance, nutrient utilization, feed efficiency, intestinal histology, and blood parameters.

Materials and methods

A digestibility trial using striping method was conducted with triplicate groups of 22 fish/tank (599.7 ± 12.9 g). Fish were fed with one reference diet and two test diets utilizing a 70:30 mixture of reference diet and test ingredients (HP or HPLO) to determine apparent digestibility coefficients (ADC) of nutrients.

In the growth trial, post-smolt Atlantic salmon (243.4 ± 6.0 g) were randomly allocated into 24 400-L tanks as groups of 35 in triplicate and fed with isonitrogenous (42% crude protein) and isolipidic (25% crude lipid) experimental diets (A-H). Soy protein concentrate (SPC) and conventional SBM were used as the primary protein sources in diets A and B, respectively. The other six diets were formulated with increasing graded levels (10, 20 and 30%) of HP (diets C through E) and HPLO (diets F through H) to progressively replace SPC. Fish were reared in a flow-through system where salinity, temperature and oxygen were maintained at optimal levels for growth and health of salmon. Fish were hand-fed experimental diets to apparent satiation three times per day. At termination (day 84), body weight was recorded, and whole-body (10 fish/tank), blood samples (5 fish/tank), livers (5 fish/tank) and distal intestines (5 fish/tank) were collected.

Results

HP and HPLO were highly digestible ingredients, with ≥92% ADCs of protein and essential amino acids. In the growth trial, final body weight ranged between 504.6-594.6 g, with HP and HPLO-fed groups (diets C–H) performing similarly to fish fed SPC (diet A). Likewise, HP and HPLO-fed groups had good feed conversation ratios (FCR < 0.9), showing a comparable or numerically better FCR compared to SPC- and SBM-fed groups. Fish fed diet B exhibited typical SBM-induced enteritis signs such as lack of normal vacuolization, increased necrosis, apparent inflammation of lamina propria and submucosa, whereas these conditions were improved in fish fed diets containing HP, HPLO and SPC. There were no statistically significant differences in final whole-body proximate composition (e.g., protein, lipid, ash and essential amino acids), hepatosomatic index as well as blood parameters (e.g., ions, glucose, total protein, hematocrit, etc). Based on these findings, HP and HPLO are considered highly digestible, nutritious, and safe protein alternatives that could be included up to 30% in salmon diet.

GUT MICROBIOTA IN ATLANTIC SALMON AFTER NUTRITIONAL PROGRAMMING WITH A VEGETABLE-BASED DIET

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Introduction

In an effort to promote sustainable aquaculture, the formulation of Atlantic salmon (*Salmo salar*) feeds has changed in the past decade, with a focus on replacing marine-based ingredients with the plant-based alternatives. The concept of nutritional programming involves initially feeding fish a specific diet type to promote successful metabolism when faced with the same or similar diet in later life. However, little is known about how these plant-based diets may impact the gut microbiota at first feeding. There is increasing evidence that initial gut microbial colonisation is essential for regulating the development of the digestive system and that these communities also aid digestion, nutrient metabolism and immune function within the gut. This study investigated the nutritional programming concept in terms of whether the initial microbiome can be permanently influenced by the first feeding diet. In our work, vegetable and marine-based diets were compared. The mechanisms by which nutritional programming occurs is not clear but there may be a contribution by microbial communities, making this study important to current understanding of the dynamic crosstalk between intestine and diet.

Materials and Methods

We investigated differences in intestine microbial communities in Atlantic salmon fed a marine rich diet (M) compared to a vegetable-based diet (V) at first feeding (Figure 1A). Samples were initially taken after two weeks of feeding either M or V (stimulus phase; Stim). All fish were then fed exclusively on a M diet and samples taken after 12 weeks (intermediate phase; Interm). Finally, fish were challenged with the V diet for six weeks and sampled (challenge phase; Chall). Whole (at Stim) and distal (at Interm & Chall) intestines were taken from 18 fish per group (six randomly sampled fish from triplicate tanks at each phase, n = 108 total). Water and feed samples were also collected at each phase. Microbial communities were assessed by 16S rRNA V3-V4 amplicon sequencing using Illumina MiSeq platform. Sequences were analysed using DADA2 pipeline to identify the microbial community as Amplicon Sequence Variants (ASVs). Functionality of the microbial communities was inferred using PICRUST2.

Results

Alpha diversity refers to the diversity within a sample, examining the microbiota structure in regard to richness (number of taxonomic groups; Chao1 index), evenness (distribution of abundances of the groups; Pielou index), or a combination of the two (Shannon index). Microbial diversity and richness was highest in the intestine at Stim (Kruskal–Wallis $p<0.001$) with a distinct and significant reduction at the Interm phase and a further decrease ($p<0.05$) at Chall. The drop in diversity was the result of an increase in relative abundance of Pseudomonas at Interm ($p<0.0001$) and Chall ($p<0.05$) phases (Figure 1B). Beta diversity refers to microbial community differences between samples or groups. Beta diversity revealed more clustering between sample types (intestine, feed and water) at each phase rather than between different phases. PERMANOVA analysis revealed less microbial homogeneity between Interm and Chall in M ($p<0.001$) compared to V ($p=0.041$). Water samples were predominantly comprised of Proteobacteria and Bacteroidota whereas Firmicutes had the highest abundance in feed. At genus level, in both M and V, *Pseudomonas* in intestinal samples increased in abundance from the Stim to Interm (Mann–Whitney $p<0.0001$) and Chall ($p<0.05$). In contrast, *Lactobacillus* decreased in abundance from Stim into Interm ($p<0.01$) and Chall ($p<0.01$) (Figure 1C). In feed, *Lactobacillus* relative abundance constitutes more than 70% of total abundance. A core microbiota was detected at ≥ 0.1% in 80% of the fish. At Stim, 14 and 10 ASVs were identified in M and V respectively, with 10 ASVs overlapping between M and V. At Chall, an unclassified genus of family Ruminococcaceae (of Phylum; Firmicutes) was present in both M and V. *Pseudomonas* sp. was additionally detected in M. There was no difference at the functional level for the Chall M vs V.

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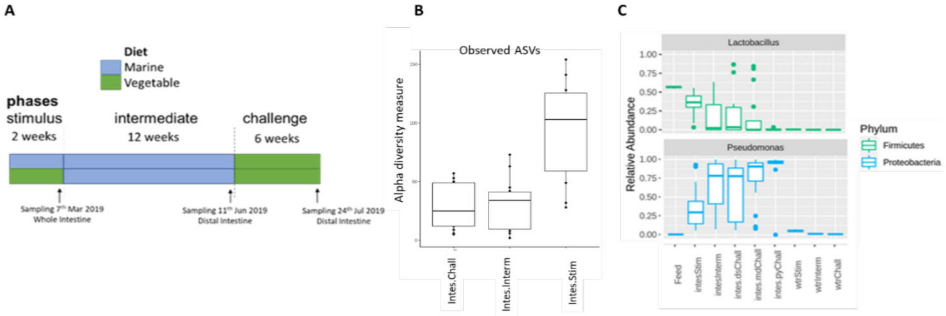

*Figure 1 **A**: Timeline of dietary manipulations with three sampling points for gut microbiota. **B**: Alpha diversity of intestinal samples across phases., **C**: Relative abundances of Pseudomonas (Firmicutes) and Lactobacillus (Proteobacteria) across sampling points and sample types.*

Discussion and conclusions

Although well-established in mammalian species, nutritional programming in fish has only relatively recently received attention. The microbial richness, evenness and diversity were highest at Stim although no difference between M and V was observed in the microbial homogeneity with the intestinal microbial community dominated by Firmicutes (mainly *Lactobacillus* sp.) and augmented by Proteobacteria. Comparison of intestine with feed and water suggested acquisition of microbiota from the environment (Firmicutes from feed and Proteobacteria from water). At Interm, richness and diversity were higher in M than V, while V was more homogenous, this may reflect the change of diet form vegetable to marine in V, while M was kept on marine during Stim and Interm. Across phases, M was microbially richer than V (mainly Firmicutes) that could imply the marine diet represents a rich medium for different microbes to be established. Interestingly, when fish were subsequently challenged with vegetable diet, there was similar microbial homogeneity between M and V. Additionally, there was comparable homogeneity and beta diversity in V at both Interm and Chall whereas for M treatment, microbiota was less diverse at Chall compared to Interm. This may suggest that fish fed V were nutritionally programmed at an earlier stage and adapted the response to V diet to be more similar in the microbial community composition to M. This was also supported by having the same core microbiota Ruminococcaceae that was observed in M and V. Moreover, there was a lower number of core microbiota in Chall compared to Stim with no statistical difference between top genera taxa in M vs V across phases (except for *Pseudomonas*). These results indicate that fish gut microbiota communities shift in a homogenous pattern with age regardless of diet and then select a more specific microbial community as the fish develops. It is worth noting that although the *Pseudomonas* sp. were not classified at Chall, we observed no negative impacts on health or functional inference suggesting that *Pseudomonas* sp. was not pathogenic.

The work was funded by BBSRC grant NUTRIPROG (BB/R018812/1) and MMT was funded by Newton-Mosharafa PhD Scholarship.

TOTALLY TUBULAR: EXPERIENCES WITH A PROTOTYPE SUBMERSIBLE MUSSEL CULTIVATION SYSTEM

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Adoption of tube-net systems by mussel farmers as an alternative technology to longline systems in northern Europe provides potential for increased biomass yields and decreased operational costs. These systems are typically designed as a 100m PE tube for support and buoyancy of a suspended net as substratum for mussel attachment. Fixed buoyancy and mechanization of harvest or thinning reduces labor requirements for farm maintenance. Controlled submersion of the system, similar to finfish cages, has gained substantial interest primarily for eliminating visibility of farm structures (social acceptance), reducing predation from molluscavorous waterfowl, and offshore production. Visibility of tube-net farms in Denmark, for example, has generated negative feedback from coastal residents and consequently a more protracted permitting process for new farms. Predation by eider ducks, related to farm visibility, has been documented to cause extreme losses of mussel biomass, if not total. Transitioning mussel production offshore, in higher energy conditions, demands more robust engineering of cultivation gear and positioning of farms in the water column below potential wave height.

In the EU Horizon 2020 Aquavitae project, we developed a prototype submersible tube and observed its behavior over two growing seasons. Mussel settlement and biomass accretion were monitored, while manipulation of the system's buoyancy was conducted to maintain a submerged position off of the sea floor. Different ballast techniques were tested in order to control the position of the system relative to biomass loads. In contrast with submersion of the conventional tube-net system, this modified tube requires substantially less ballast and has potential for some automation in buoyancy control. We present the tube design, experiences managing the system, and lessons learned.

MUMIFAST: MUSSEL MITIGATION FEEDS AND SUPPLY SYSTEM TECHNOLOGICAL DEVELOPMENT

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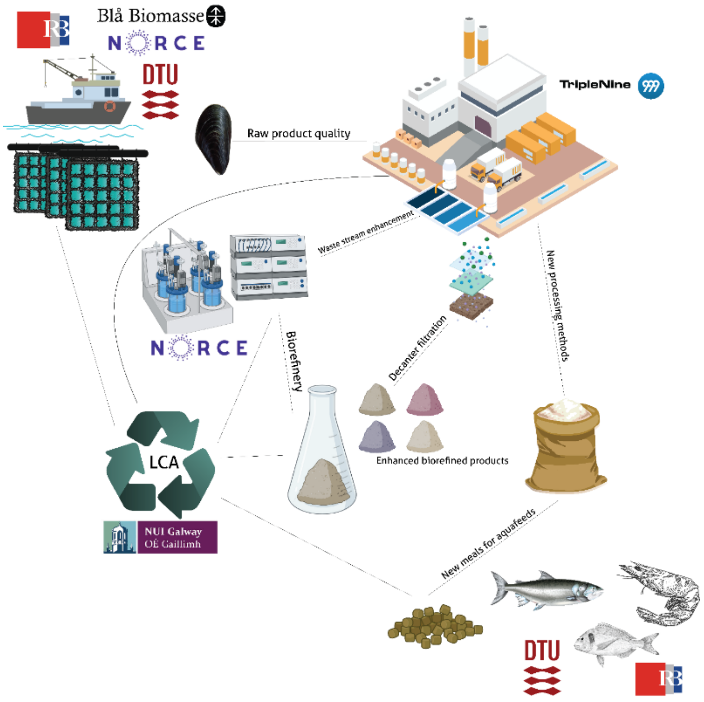
The MuMiFaST project gathers European expertise on the value chain related to undersized non-uniform mussel biomass compared to mussels produced for human consumption. As either a new industry utilizing undersized mussels, or as one focused on maximizing production of heterogeneous mussel biomass, this marine bioresource can be produced sustainably, and exhibits promise in use as a high-quality animal feed and in nutritious value-added food products. The primary needs for innovation in this industry are related to post-harvest preprocessing and processing of large volumes of heterogeneous mussels to a quality standard suitable for the aquaculture feeds industry. In this project the partners will develop advancements in the supply system of blue bioeconomy value chains of mitigation mussels between TRL3-6, providing proof of concept of new perspectives on waste reduction and utilization of the entire biomass towards production of highly quality ingredients for the aquaculture feed industry. Application of this undersized mussel model in a wider European context will be explored through the partner organizations and broad stakeholder engagement.

A brief introduction to the project and its work packages is presented.



THE EUROPEAN PERCID FISH CULTURE CORE GROUP AS A KNOWLEDGE SHARING GROUP

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In 2012 the European Percid Fish Culture (EPFC) was founded as a thematic group within the European Aquaculture Association (EAS). This EPFC was mend to bring researchers in contact with their target group, being the upcoming pikeperch (*Sander lucioperca*) and European perch (*Perca fluviatilis*) producers. From the founders' point of view, research needs to solve, clear-out, the bottlenecks in the industry and to push this new sector forward.

The fist EPFC workshop was held in 2012 during the EAS congress in Prague, Czech Republic. Year by year the network of researchers and fish producers expanded, and the contacts and exchange of knowledge and insights became more valuable. All with one common topic: percid fish culture. In 2017 the idea grew to evolve from an knowledge exchanging platform towards a knowledge sharing group.

Beginning 2019 the EPFC Core Group was founded as a knowledge sharing group within the EAS thematic group EPFC. All 26 members paid a membership fee and signed a cooperation agreement for 3 years. A Short overview of the first 3 years activities and future activities is given in the presentation.

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THE SMALL DIFFERENCE - PRACTICAL IMPLICATIONS OF PH ON TARGET PLANT NUTRIENT CONCENTRATIONS DERIVED FROM HYDROPONICS IN AQUAPONICS

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Introduction

In aquaponics, imbalances in concentrations of plant nutrients are commonly reported. Supplementation of nutrients is suggested to balance the nutrient profile of water in a way that it resembles nutrient solutions used in hydroponics. However, studies within this context found limitations in the manipulability of some nutrients (Shaw et al., 2022). A key difference between aquaponics and hydroponics is the maintained system pH, among other parameters. Considering the importance of the pH for the solubility of a broad range of salts (Sambo et al., 2019), the question must be raised whether nutrient concentrations targeted in hydroponics can be reached under aquaponic conditions. This work intends to provide a first insight into the implications arising from the pH as master variable, determining solubility of poorly soluble salts in different aquaponic systems.

Material and Methods

Nutrient concentrations reported for the Hoagland plant nutrient solution were used for further calculations (Resh, 2012). A pH of 6.0, 6.8 and 7.5 and a CO_2 partial pressure of 0.054 atm was assumed for hydroponic, coupled, and decoupled systems, respectively. Concentrations of CO_3^{2-} and PO_4^{3-} species were calculated using the target concentrations stated in the formulation of the nutrient solution as total concentrations. Resulting maximum solubility of the cationic plant nutrients forming poorly soluble salts was calculated and target concentrations of the cations were eventually divided by the theoretical maximum solubilities to obtain maximum solubility satiation levels.

Results and Discussion

According to the calculated theoretical solubility of calcium, $Ca_3(PO_4)_2$ is exceeding the maximum solubility in all three systems. This could be due to interactions between calcium and other ions that are not covered by the simplistic approach used. On the other hand, the low system pH reduces the carbonate concentration in hydroponic systems to a level where precipitation of calcium carbonate does not occur. The threshold concentration is exceeded at higher pH as present in coupled and decoupled aquaponics. This might explain why calcium deficiencies are reported in aquaponic systems (Goddek et al., 2019). Magnesium solubility ranges at levels where target concentrations for optimum plant growth are unaffected. Recommended concentrations for iron are higher than the solubility of poorly soluble iron salts such as iron phosphate. While exceeding the calculated threshold concentration in hydroponics is possible by addition of iron in complexed form (e.g., FeEDTA), the same effect can be achieved by interactions of iron cations with dissolved organic matter. A study has shown that the addition of fulvic acid has the potential to mobilise iron (da Silva Cerozi, 2020). However, studies showing the feasibility of adding increased amounts of fulvic acids in aquaponics have not been conducted until now.

The maximum solubility copper might be exceeded in decoupled aquaponics, with precipitation of copper phosphate taking place within the aquaculture unit if copper is excreted as part of feed metabolization or direct addition. This could lead to losses of copper if precipitation occurs at inaccessible places such as the inside of the pipes. Manganese and zinc, on the other hand, might not be affected by increasing pH.

Overall, the results clearly show that the pH could be a causative factor for nutrient imbalances in aquaponic systems, allowing higher nutrient concentrations in hydroponics. However, smart nutrient management could reduce nutrient losses and increase the efficiency of aquaponic food production.

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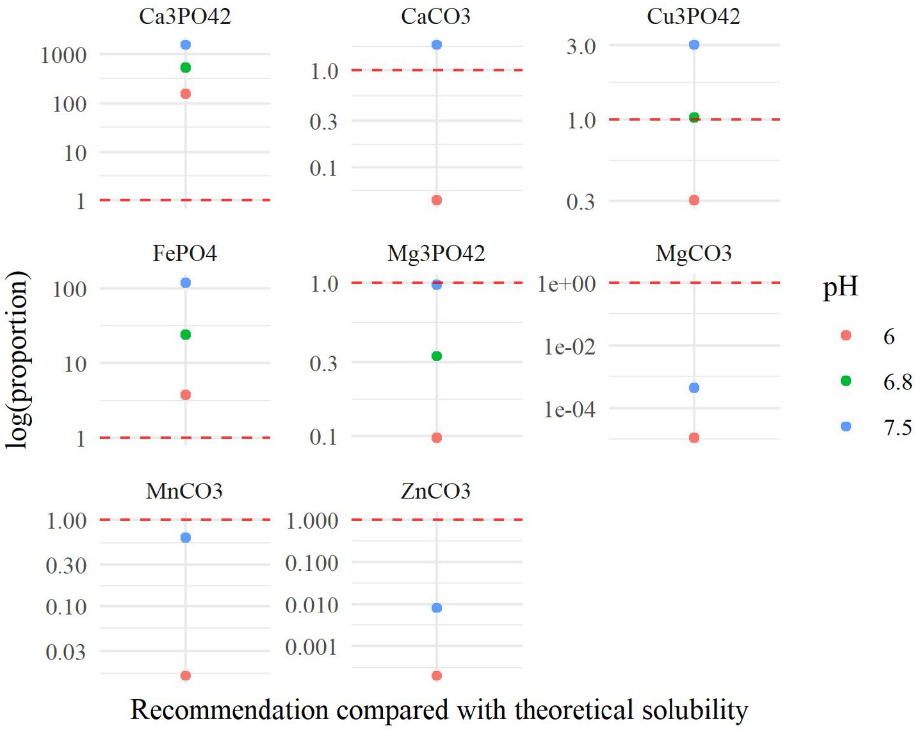

Figure 1: Percentage satiation levels of some cationic plant nutrients in dependence of the solubility of their poorly soluble salts. Dashed red line indicates 100% saturation.

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EVALUATION OF POTENTIAL DISRUPTORS OF INTESTINAL HOMEOSTASIS IN GILTHEAD SEABREAM JUVENILES

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Introduction

Gut health plays a key role in fish nutrition since it is closely associated with an effective nutrient uptake and with the modulation of fish immune system and antioxidant status. A disruption in the fish intestinal permeability and homeostasis may result in reduced growth and feed efficiency, lower resistance to stressors and diseases, and overall poorer health status.

Soy saponin (SAP) and dextran sodium sulphate (DSS) have been shown to provoke mild to severe intestinal inflammatory processes in fish and mice, respectively. This study aimed to assess these compounds as potential disruptors of intestinal permeability and homeostasis in gilthead seabream juveniles by evaluating the intestinal response to different doses of SAP and DSS at 24, 48 and 72 h. Moreover, the expression of genes coding for antioxidant response (catalase – *cat* and glutathione peroxidase – *gpx*), paracellular permeability (occluding – *ocl*, claudin 12 – *cldn12* and tight junction protein 2 – *tjp2*), and immune response (cyclooxygenase 2 – *cox2* and immunoglobulin M – *IgM*) was evaluated to identify molecular markers that highlight homeostatic disruptions in the gut.

The validation of an experimental model causing acute gut inflammation and dysbiosis will allow the evaluation of functional feeds and ingredients as potential promoters of gut health. Furthermore, the validation of such model would be a step forward to investigate the underlying physiological and molecular mechanisms involved in fish intestinal recovery and health.

Materials and Methods

Gilthead seabream juveniles with an average body weight of 152 ± 22 g were sedated with 2-phenoxyethanol (300 ppm) and fed gelatine capsules by the "assisted-feeding" technique. This method ensured that the capsules reached the fish stomach after being gently pushed through the mouth with a hollow tube. Fish were fed capsules containing SAP or DSS at different doses according to the experimental treatment: CTRL – fish fed empty gelatine capsules; SAP1, SAP2, SAP3 – fish fed gelatine capsules containing soy saponin at 125, 350 or 700 mg, respectively; DSS1, DSS2, DSS3 – fish fed gelatine capsules with 125, 350 or 700 mg of dextran sodium sulphate. All fish were fed one capsule, except fish fed the highest doses of SAP and DSS (SAP3 and DSS3), which were fed two capsules. After the assisted-feeding, individual fish were placed in clean and aerated seawater to eliminate any residual anaesthetic and monitored for eventual capsule regurgitation. Once recovered, eighteen fish per treatment were transferred to two 100 L cylindric-conical tanks (density: 13.7 Kg m^{-3}; temperature: 16 ºC; salinity: 32 psu). Details on the experimental design are presented in Figure 1.

At 24, 48 and 72 h after capsule ingestion, the anterior and posterior intestine were sampled from six fish per treatment for gene expression, histology, and microbiome analyses. The differential expression of the selected molecular markers was evaluated. Data was subjected to principal component analysis (PCA) using the prcomp R function in the auto-scaled matrices to find potential clusters of observations. Score plots were generated for the two first principal components using the ggbiplot and factoextra packages for the open-source software R version 4.2.1 (R Core team).

Results and Discussion

DSS3 fish had a mortality rate of 88.9 %, indicating that the ingestion of the highest dose of DSS (700 mg) led to irreversible injuries in fish intestine. Therefore, the ingestion of DSS at 700 mg would not be a suitable candidate to validate this novel technique. Conversely, no mortality was observed in the remaining treatments over the 72-hour trial.

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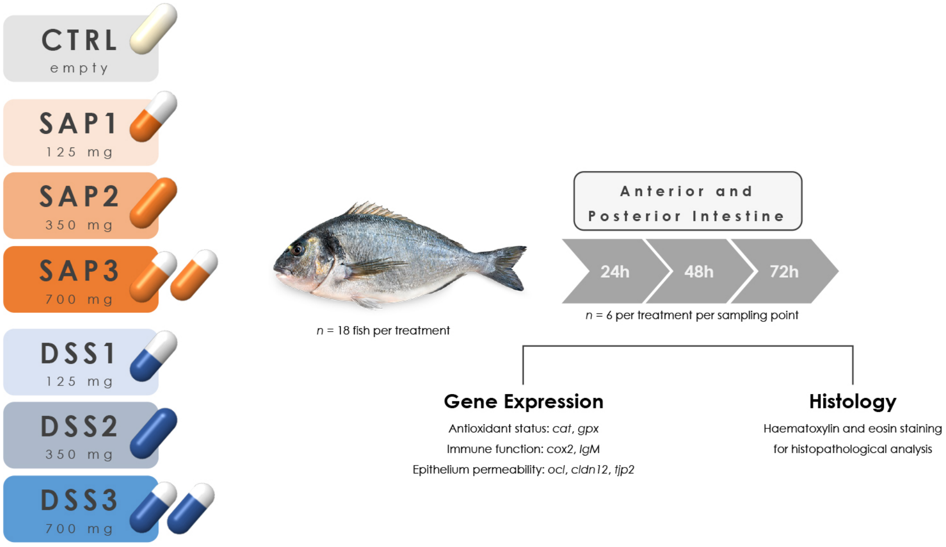

Figure 1. Experimental design.

Histology of anterior intestine sections of all sampling points, in addition to gene expression data from the 24-hour sampling, are currently being evaluated. Furthermore, posterior intestine sections will also be analysed and compared with the results obtained for the anterior intestine.

Gene expression data was generated for the anterior intestinal sections of fish at 48 and 72 h after ingestion of the gelatine capsules containing the different compounds at different doses. After 72 h, fish from the SAP3 treatment presented a differential response revealed by PCA, being the differential expression of *gpx*, *tjp2* and *cox2* the main loadings responsible for clustering SAP3 observations.

Preliminary results suggest that after 72 h, the oral administration of 700 mg of soy saponin may provoke disruption of intestinal homeostasis in gilthead seabream. This novel technique may be viable to evaluate the potential role of functional feeds as gut health promoters.

Acknowledgments

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SAFE OPERATIONS IN AQUACULTURE

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Introduction

Risk in aquaculture operations has been described according to five dimensions: risks to material assets, personnel, fish welfare and health, environment, and food safety (Yang, Utne and Holmen, 2020)irregular and often high winds, sheer remoteness, and limited weather window amplify the hazards during aquaculture operations. This paper presents a methodology for identifying hazards in aquaculture operations that take into account risks to personnel, material assets, the environment, fish welfare and food safety. The methodology considers the marine fish farm, its support service systems, external agents impacts, potential environmental disturbance and the likelihood of hazard interactions, and fish in the cage. The methodology has been used to analyse a net-cleaning operation, and the results show that it provides a good overview of hazards in a specific operation. The operator can have a better understanding of the nature of the operation to be better prepared for what can go wrong. The paper concludes that the risk assessment framework for aquaculture operation needs to be holistic to take into account multiple dimensions of risk, and the proposed methodology serves a good basis to develop the framework further. The paper also brings up the necessity to investigate further the definition of major accident and major accident hazards in aquaculture, especially for exposed fish farms that are exposed to different hazards comparing to the coastal fish farms today.","container-title":"Safety Science","DOI":"https://doi.org/10.1016/j.ssci.2019.09.021","ISSN":"0925-7535","page":"430-450","title":"Methodology for hazard identification in aquaculture operations (MHIAO. Workers at sea-based fish farms must consider all these risk dimensions in their everyday work. The occupational injury and fatality rate in Norwegian fish farming is high compared to other industries. On average, there has been one fatal accident per year in the period 2012-2020. Common modes of injuries include falls, blow by objects, entanglement/crush as well as cuts. Manual tasks are part of the work, and a survey showed that strain injuries was a main reason for worry, and the primary cause for work-related sick leave among aquaculture workers.

To support safe operations in aquaculture, safety management is mandatory. This presentation provides knowledge about the status of safety management and suggests improvements for occupational health and safety.

Materials and methods

The findings presented here are based on interviews, workshops, surveys, and document studies conducted over a period of almost ten years. Key projects include SFI Exposed (grant no. 237790), and Safer operations and workplaces in fish farming (grant no. 254899) supported by the Research Council of Norway.

Results

The focus on safety management from the regulatory authorities has expanded in recent years. The change from detailed, specific safety rules to functional regulatory requirements has increased the number of company-internal procedures (Størkersen et al., 2020). Aquaculture managers and operative personnel experience improved systematic safety work, but also that safety management systems could be more integrated in daily work (Kongsvik et al., 2018). Findings show that internal procedures are seen as too complicated, and that operators are not involved in the analysis phase of risk assessments (Holmen, Utne and Haugen, 2018). Challenges also exist regarding the implementation of risk assessments in the organizations and the transfer from risk assessments to improved operational safety.

Safety management that aims to protect workers must also consider the other risk dimensions found in operations, as well as the regulatory framework of the industry. Conflicting objectives related to protection versus production are found in aquaculture operations (Størkersen *et al*., 2021), and a survey showed that some employees think that production is prioritized at the expense of safety (Kongsvik *et al*., 2018). This may be related to organizational factors such as long work shifts and heavy workloads during large operations, which in turn may be connected to regulatory requirements.

Reduction of salmon lice and prevention of escape remain the major risks that guide regulation and technology development Salmon lice is the only indicator for sustainability and guides growth through the traffic light system. Delousing operations thus become highly important to make sure lice numbers are below the limit, but these operations may increase the risk levels personnel, fish welfare and environment negatively. A survey showed that during delousing, personnel report that they may work up to 20 hours for several days in a row (Thorvaldsen et al., 2020). Delousing has also been identified as an operation where risk of escape, lower fish welfare and fish mortality increases (Føre and Thorvaldsen, 2021).

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Practitioners experience an abundance of internal rules and protocols due to mechanisms such as work auditability, managerial insecurity and liability and audit practices. It is therefore important to avoid implementing measures that increase the distance between how we think work is done, and how it is actually done. Safety management requires procedures, resources and involvement of personnel, and the practical value depends on the communication between those who develop the system and those who use it. Research confirms that safety measures that fit well with the everyday reality of workers are generally considered to have a higher practical relevance than others. For instance, the perceived practical relevance of measures such as planning (to avoid long working hours and alone work), involving personnel and a ensuring a skilled team, is considered to be higher than procedures (Thorvaldsen *et al.*, 2021).

Conclusion

To ensure adequate safety management it is important to consider how operations are conducted. Safety activities should be assessed through involvement of personnel at the fish farms who have detailed knowledge of work practices. This way all risk dimensions and how they affect occupational health and safety can be considered.

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A CROSS REGIONAL STUDY OF DRIVERS FOR INNOVATION IN AQUACULTURE

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Introduction

Norway is a leading producer and exporter of Atlantic Salmon (*Salmo salar*) globally. Open cages in the coastal zone remain the most common type of production system, but new production systems for open ocean aquaculture, and closed production cages in sea or land-based sites are also emerging. There are multiple reasons for the development in the Norwegian industry. Environmental challenges have resulted in regulatory changes over the past decade. The Norwegian government implemented several measures to make the aquaculture industry more sustainable, especially focused on environmental sustainability. Important initiatives include regulations that aim to spur innovation to reduce environmental impact and thus, enable industry growth. Key drivers for technology development in Norwegian aquaculture include prevention of fish escape, reduction of salmon lice levels in the fish farms and utilisation of new areas further from shore (open ocean aquaculture) or on land. To promote technological innovation, development licenses were introduced by the government in 2015 (Føre et al. 2022).

In the project DEVELOP, innovation in aquaculture resulting from development licenses was studied. This research was funded by the Research Council of Norway (grant number 301486). We also investigated drivers for innovation beyond the Norwegian regulatory context. To compliment the impacts to innovation of Norway's development licenses we investigated and compared similarities and differences in the observed/present drivers for innovation in aquaculture industry across nations and regulatory contexts.

Materials and methods

The international advisory board for the project DEVELOP conducted an online workshop to compare and discuss current technology status and drivers for innovation in different geographical regions. This comparison was based on the participants own experiences and as well as literature reviews.

Results

In Canada, drivers for innovation are geographically variable and are substantially different between the west and east coasts of the country. While all operators face challenges associated with climate change and warming waters, sea lice and disease, and mass die offs, the social drivers for innovation are more intense on the west coast where licences are being phased out and where there is strong pressure to shift to land-based systems of production. On the East Coast, producers enjoy the support of provincial governments and so social opposition plays a somewhat smaller role in driving innovation.

In Japan, environmental problems and more use of unused sea areas are key. Environmental problems are regulated by Sustainable Aquaculture Production Assurance Act. Most cages are in sheltered areas, but several offshore and submergible cages projects have been tried.

In USA aquaculture is regulated through environmental laws. Identifying suitable geographic areas called Aquaculture Opportunity Areas is ongoing. Identifying and mitigating environmental, economic, social, and cultural considerations as a part of an Environmental Impact analysis are a part of this process.

In the UK strategic plans for increasing growth exist. For salmon transparency in sea lice numbers and mortality is required. Innovation drivers include fish health and welfare, environmental sustainability, final product quality, diversity and inclusiveness, HSE and social license to operate.

In New Zealand, shellfish and seaweed structures for exposed sites are being developed. Innovation drivers include sustainability, efficiency, reduced negative impact on surroundings, animal welfare, utilization of exposed sites and development of equipment for exposed areas as well as social upliftment of low-income areas.

(Continued on next page)

Conclusion

When comparing the regions, there are several overlapping challenges driving the innovation in aquaculture. Still, the political ambition for the industry to grow is more prominent in Norway than the other regions. The regulatory scheme based on production licenses, which are limited and have considerable value, equip the Norwegian authorities with an effective policy tool (the possibility of allocating new licenses) to spur technological innovation by providing risk-relief and increased production capacity to actors that bring about technological innovations. Hence, a key attribute for policy to spur innovation is the licence value and the existing competition for increased allowed production capacity. Common drivers across nations are related to the need to reduce environmental impacts, the need for area (sheltered and less sheltered), need for economic development, and the need for social license. Many of these are related, however, the regulatory contexts varies and as a result, there are different implications for innovation and how these drivers for innovation will affect industry development and regulation.

Social license is pointed out to be an important driver for innovation as specific environmental challenges affect the social acceptability of the industry. Although not stated explicitly, this is an important underlying cause for governmental and industry actors when deciding on regulations and measures for innovation. Further investigation of social implications of innovation in aquaculture could be fruitful for understanding the possibilities of regulatory measures targeting technological innovation in aquaculture.

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OVERCOMING THE INFERTILIY PARADOX TO PRODUCE INFERTILE GENOME-EDITED FISH

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Genome Edited (GE) fish are already available in some markets and as permissive legislation becomes more common they will be available elsewhere. GE will be used by established breeders to bring sustainable benefits in health, welfare and efficiency. GE can improve traits that were previously difficult to handle such as infertility, sex, disease resistance, efficiency and yield. GE alterations are indistinguishable from natural mutation. Breeders will make edits that have large effects on traits that improve adaption to farm environments but may reduce fitness in the wild. Most fish farming happens in an open environment and escapees could interbreed with wild counterparts. Fish that have been genome edited for improved commercial performance should be made infertile to prevent introgression of new gene variants into the surrounding environment.

The infertility paradox where breeders need fertile broodstock and producers need infertile production fish makes it challenging to breed for infertility. This paradox can be overcome if desirable genotypes can be made infertile by GE and their germ cells transferred to surrogate broodstock for multiplication. Using this reproductive strategy, GE of functional traits in high merit fish can be combined with GE for infertility and surrogate broodstock used to produce large volumes of infertile juveniles for production. Infertility is integrated into the breeding programs to ensure ecological sustainability and prevent movement of GE alleles for improved farm performance into wild populations.

GENOME EDITING TO PRODUCE MONOSEX AND STERILE FISH FOR AQUACULTURE

John Buchanan, Xavier Lauth, Takeshi Umazume and Alan Tinch*

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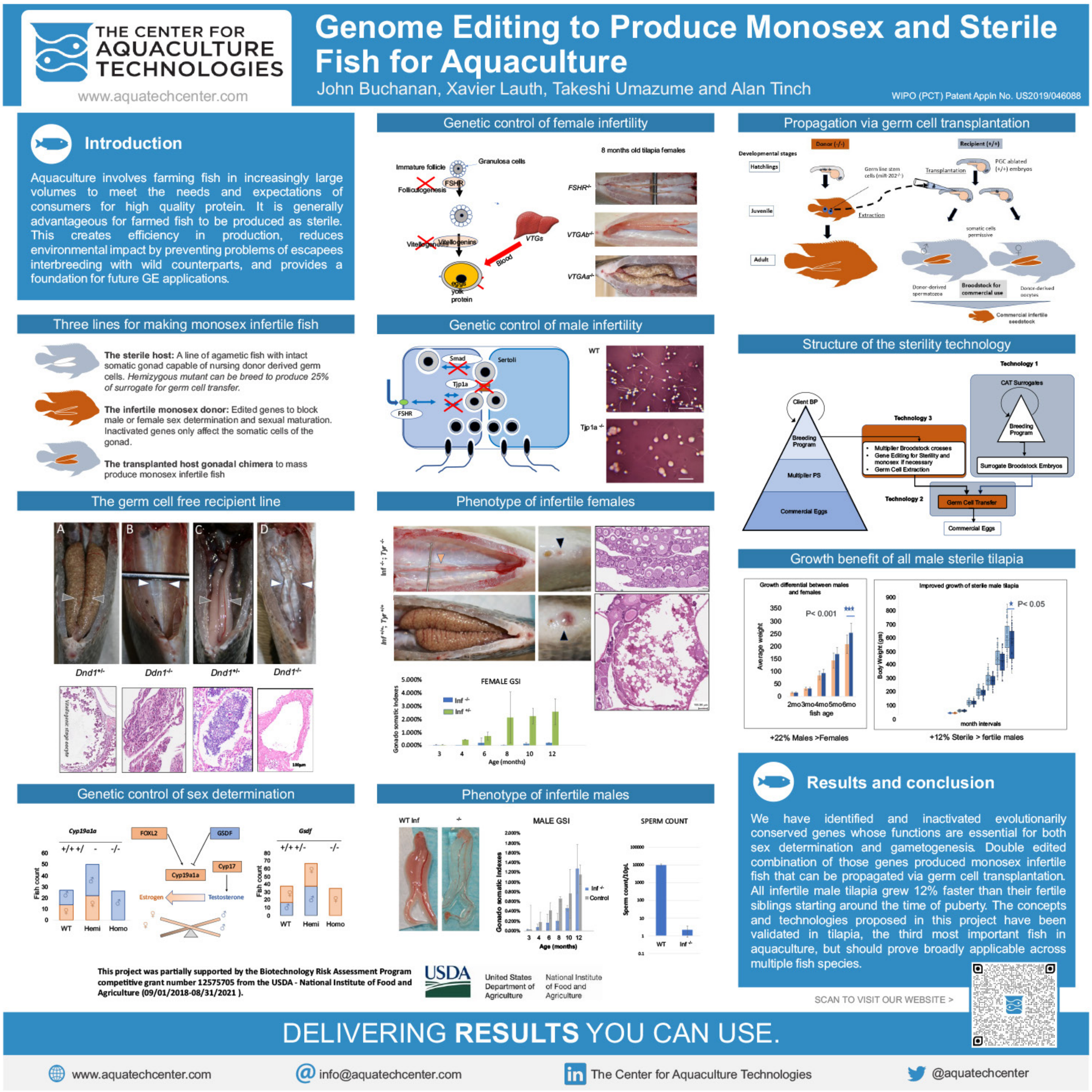

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Genome Editing to Produce Monosex and Sterile Fish for Aquaculture

John Buchanan, Xavier Lauth, Takeshi Umazume and Alan Tinch

WIPO (PCT) Patent Appln No. US2019/046088

Introduction

Aquaculture involves farming fish in increasingly large volumes to meet the needs and expectations of consumers for high quality protein. It is generally advantageous for farmed fish to be produced as sterile. This creates efficiency in production, reduces environmental impact by preventing problems of escapees interbreeding with wild counterparts, and provides a foundation for future GE applications.

Three lines for making monosex infertile fish

The sterile host: A line of agametic fish with intact somatic gonad capable of nursing donor derived germ cells. *Hemizygous mutant can be breed to produce 25% of surrogate for germ cell transfer.*

The infertile monosex donor: Edited genes to block male or female sex determination and sexual maturation. Inactivated genes only affect the somatic cells of the gonad.

The transplanted host gonadal chimera to mass produce monosex infertile fish

The germ cell free recipient line

Genetic control of sex determination

Genetic control of female infertility

Genetic control of male infertility

Phenotype of infertile females

Phenotype of infertile males

This project was partially supported by the Biotechnology Risk Assessment Program competitive grant number 12575705 from the USDA - National Institute of Food and Agriculture (09/01/2018-08/31/2021).

USDA United States Department of Agriculture National Institute of Food and Agriculture

Propagation via germ cell transplantation

Structure of the sterility technology

- Multiplier Broodstock crosses
- Gene Editing for Sterility and monosex if necessary
- Germ Cell Extraction

Growth benefit of all male sterile tilapia

+22% Males >Females

+12% Sterile > fertile males

Results and conclusion

We have identified and inactivated evolutionarily conserved genes whose functions are essential for both sex determination and gametogenesis. Double edited combination of those genes produced monosex infertile fish that can be propagated via germ cell transplantation. All infertile male tilapia grew 12% faster than their fertile siblings starting around the time of puberty. The concepts and technologies proposed in this project have been validated in tilapia, the third most important fish in aquaculture, but should prove broadly applicable across multiple fish species.

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IMPACTOFPHOTOPERIODE,TEMPERATUREANDFEEDINGREGIMEMANIPULATION ON ATLANTIC SALMON, *Salmo salar,* FRESH WATER GROWTH PERFORMACE AND GENE EXPRESSION

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Introduction:

Atlantic salmons early and freshwater developed is regulated by both endogenous and exogenous environmental ques (Steffansson et al., 2008). Commercial production often manipulates the culture environment to both increase production and its flexibility (Bjornsson et al., 2000). Use of land-based systems, such as recirculating aquaculture systems (RASs), are now increasing in use in order to maintain productivity gains while mitigating negative effects to the wider environment (FAO, 2017). However, the environment provided in RASs can have heightened stability lacking variation know as important to salmon's development. The accumulated effect of the RASs specific environment is therefore hypothesised as contributing to the poorer performance of fish produced in these systems later in life. We therefore aim to investigate the potential negative impacts of common husbandry manipulation and lack of environmental entrainment of RASs. Specifically, the (possibly interacting) effect of photoperiod, temperature and feeding stability on *s.salar* and how this has differently impacted on gene expression and growth performance.

Materials and Methods:

The fish originated from a stock housed at Buckie burn facilities, University of Stirling. Approximately 2,500 were transferred to temperature aquarium at the University of Stirling at 15g and held under constant light, constant feed and 11.5 degreesC. Whole brain tissue samples were taken at baseline (n=6). Fish were pooled and split between six treatment groups for 800dd, each run in triplicate. Treatments were a combination of photoperiod, feeding, and temperature regimes (see table 1). At 800dd whole brains were sampled from 6 fish per treatment (n=36). Feed intake was assessed for the two weeks following final sampling. RNA was extracted from all samples and sequenced by Novogene on NovaSeq 6000 PE150 platform. Three libraries were removed from further analysis due to high duplication rate (52.6%, 63.3% and 64.7%). Libraries cleaned using fastp and aligned against *s.slara* genome (Ssal_v3.1, RefSeq GCF_905237065.1) with hisat2 and Stringtie. Using DESEq2 differently expression genes (DEG) were identified, with p<0.001 and –logfold2 change >2 thresholds. DEG genes were identified relative to control conditions, accounting for DEG through time (baseline compared to 800dd control).

Results and Discussion:

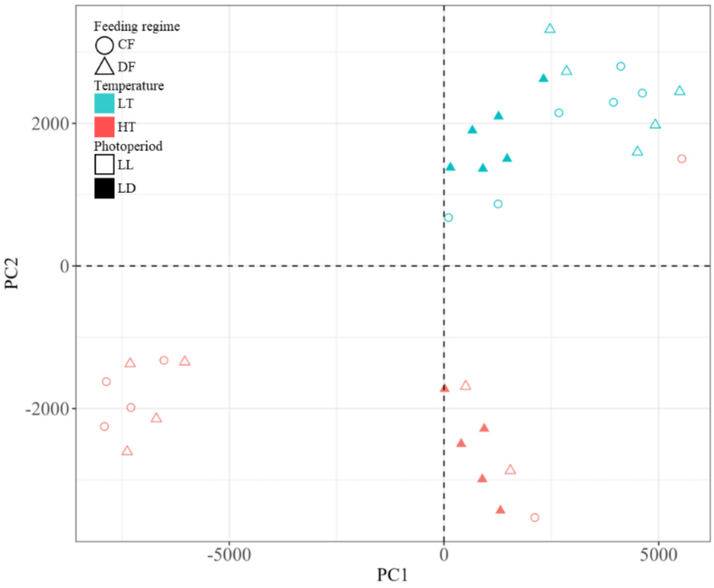
Temperature had the greatest impact on fish growth, with low temperature treatments on average gaining 49.54g (SEM: 2.26) over 800dd, 16.64g more than those held at 15.5degreesC. While fish exposed to continuous light and continuous feed showed greater weight gains, they were not significantly greater than other treatment groups. Interactions between environmental treatments reiterated these trends. Considering all treatments (table 1), significant differences in growth were only seen when comparing treatments at low temperatures, where the best performing group was under continuous light and feed (gaining 52.90g, SEM 1.61), in line with other findings. Principal component analysis also indicated temperature as an impactful factor, with low and high temperature treatments clustering separately along PC2. Photoperiod regime also appeared to have a secondary impact on samples along PC1, with only high temperature groups differentiating along PC1 (figure 1). DEG due to photoperiod, temperature and feeding regimes, relative to the control were identified. Results indicated that temperature maybe principally driving changes in gene expression, whereby relative to low temperature control, a higher number of DEG were seen in treatment groups held at 15.5degreesC, for example across the six treatment groups no DEG were identified in those held at low temperature relative to the control.

In summary, in terms of growth low temperature, continuous feed and continuous light produced the best performing fish. Out of these treatments, temperature appears as having the greatest impact on gene expression in Atlantic salmon brains, with secondary impact of photoperiod, acting particularly at high temperatures despite no difference seen in their growth. Continual analysis will identify the function of DGE as well as these samples methylation patterns to further understand the implications the RAS environment.

(Continued on next page)

Table 1: Treatment groups, comprising of continuous light: LL or 18:6 day night cycles: LD, continuous feed: CF or feeding in the perceived day: DF and temperature of 11.5 degreesC: LT or 15.5degreesC: HT. Control conditions were constant light, constant feed and low temperature.

Photoperiod	Feeding regime	Temperature
LL	CF	LT
LL	DF	LT
LD	DF	LT
LL	CF	HT
LL	DF	HT
LD	DF	HT



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AN AFFORDABLE AND EASY-TO-USE TOOL FOR AUTOMATIC FISH LENGTH AND BIOMASS ESTIMATION IN MARICULTURE

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Introduction

Aquaculture provides two-thirds of the world's aquatic products. It represents a source of high-quality protein for humans and has become one of the fastest-growing industries in global food production. Along with the world population increase, global aquaculture production is expected to increase swiftly to meet the fish demand for human consumption in the forthcoming years [1]. Indeed, the rapid rise of the aquaculture production system has led to several issues, such as fish over-feeding, disease outbreaks, and water pollution. For this reason, the industry and academic community are requested to seek strategies to increase productivity and efficiency of the aquaculture systems, while controlling the negative impact [2]both for production systems and for breeding programs. Most of the current methods are based on manual measurements, which are laborious and stressful to the animals. Therefore, the development of fast, precise and indirect measurement methods for traits such as body weight (BW and improving the environmental, economic, and social sustainability. In this framework, automation and smart technologies are gradually gaining traction in the sector. Precision aquaculture applies control-engineering standards to fish production, to improve farmers' monitoring, control, and recording of biological processes in fish farms [3]. The management of land-based and offshore aquaculture facilities requires an accurate periodic estimation of fish length and weight to control the growth of fish, allowing for calibrating feed administration and evaluating animal welfare. Length and biomass estimation is usually conducted manually on a reduced subsample. Here we present a low-cost prototype system ("smart buoy") tested in a commercial mariculture cage. The buoy, equipped with stereoscopic cameras, can capture fish images, which are then automatically analyzed using the cognitive capabilities of AI and computer vision algorithms. This technology allowed achieving precise automatic length and weight estimation of cultured gilthead seabream (*Sparus aurata*, L.) during the on-growing period.

Materials and methods

The study was conducted at the "Maricoltura e Ricerca Soc. Coop." fish farm (Capraia Island, Italy), approximately 0.17 nautical miles from the coast. The underwater stereo camera was installed on a floating buoy anchored within a commercial-scale farming cage. The two-camera lenses were spaced 8 cm apart on the vertical axis and oriented towards the cage net at about 3 m. The buoy transmitted over a mobile network to a cloud-based site where images were stored. Images capturing and analysis were carried out during the last day of the commercial finishing phase, in a cage that hosted about 3500 gilthead seabreams. For fish body length estimation, a complex AI pipeline was designed; the pipeline has been split into smaller packages to break down the final task into its components and thus simplify and manage the analysis more efficiently. The raw stereo images were fed to an improved Convolutional Neural Network (CNN) called YOLOv4 [4]and theoretical justification of the result, is required. Some features operate on certain models exclusively and for certain problems exclusively, or only for small-scale datasets; while some features, such as batch-normalization and residual-connections, are applicable to the majority of models, tasks, and datasets. We assume that such universal features include Weighted-Residual-Connections (WRC, trained to locate the single fish in the image through the use of bounding boxes. In a second step, each bounding box was used to obtain the individual image of the fish and then entered into a CNN, RESNET-101 (RES101) [5], optimized for image recognition. From each image, the relevant individual fish were extracted and annotated with two reference points (landmarks), which allowed the system to measure the fish standard length in pixels by connecting the two marks. Finally, the length unit was transformed from pixels to centimeters, using the translation information derived from the calibration phase with the chessboard target.

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A total of 190 fish (from catches intended for sale) were also collected and photographed in controlled conditions (digital scale, tripod, and professional camera). The data obtained from this subsample were used to *i)* compare biometric results obtained from AI; *ii)* calculate a length-to-weight relationship (LWR) curve, and *iii)* deduce fish side view area (in cm^2). To improve the biomass calculation performance, not derived by the estimated standard length (SL), a new approach was also developed to infer the weight of the fish from its side view area. Here we present the preliminary methodological results based on Mask R-CNN, a framework for the segmentation of object instances that will be exploited for the automatic extrapolation of the area [6]. Moreover, to fit a model fitting between natural log-transformed body weight and side area (RANSAC algorithm [7]Random Sample Consensus (RANSAC), a total of 190 seabreams were weighed and photographed in controlled conditions: 110 from the same cage equipped with the smart buoy and 80 from a land-based experimental farm in Italy. The Mean Absolute Percentage Error (MAPE) was used to measure the accuracy of the prediction between the measured weight and the estimated weight from the side area of the fish.

Results and Discussion

The AI algorithm identified 272 fish from 76 shoal images taken within the cage. Of the total, 148 fish were discarded as the translation rate fell below a threshold of 140 pixels. The remaining 124 fish were automatically processed for standard length estimation (AI measurements). The mean standard length and weight (± SD) of fish obtained from sampling and those estimated by the AI were respectively 27.1 ± 1.6 cm and 26.3 ± 4.4 cm, and 606 ± 103 g and 585 ± 239 g, with mean differences for length and weight of 3.0 and 3.6%. Both distributions behaved as normal curves (Shapiro = 0.9, $P = 0.07$; Shapiro = 0.99, $P = 0.59$, for sampled and AI estimated, respectively), although variances were not homogeneous (Levene test $P < 0.0001$). However, the most representative classes (26, 27, and 28 cm bins) were found to be the same from both actual measurements and AI. The distribution estimated by the AI had greater dispersion than the sampled one, which was more clustered. On the other hand, the means were not significantly different ($F = 3.90$, $P = 0.05$).

Preliminary results concerning a novel automated approach to estimate the biomass of a single fish from its side view area have shown promising results: the RANSAC algorithm significantly fitted between seabream measured weight and estimated area (in cm^2) (calibration dataset n = 127; equation: $y = 1.58x - 2.05$; $r^2 = 0.945$, $P < 0.0001$). MAPE (±SD) between measured vs. estimated weight reached 1.26 ± 1.55 % (validation dataset: n = 63). As reported by previous studies [8], [9]500 weights and corresponding images of harvested Lates calcarifer (Asian seabass or barramundi, there is a strong positive relationship between weight and side view area in several fish species. We believe this evidence can be used to deduce the single fish biomass starting from data obtained by an AI approach independent of the estimated length. In this way, more accurate results would be found compared to those obtained through the traditional length-to-weight relationship, a method commonly used but strongly influenced by the rearing site and which involves important estimation errors.

Computer imaging together with artificial intelligence perfectly meets the needs of a modern European sustainable aquaculture by optimizing operational activities and decreasing operating costs. The buoy developed in this study, despite being a prototype, gave accurate results (3% of the fish standard length between AI and sampling measurements, along with a very similar size class distribution), proved to be viable in commercial mariculture, and has a moderate cost (about €3,000) compared to other solutions on the market. Further steps will cope with the improvement of the buoy system and the quality of the stereo photos. These upgrades will allow for better monitoring of farmed fish characteristics, in particular biomass estimation, and will allow the device to be applied to other types of farming, such as land-based aquaculture (raceways, ponds, and tanks), and different fish species.

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BIOCHEMICAL, GENETIC AND HISTOLOGICAL CHARACTERISTICS OF WILDLIFE AND AQUACULTURE PIKEPERCH

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Introduction

Pikeperch (*Sander lucioperca*) is characterized by rapid growth, high breeding performance, and its fillet is popular among consumers and therefore in great demand on the national and international markets. As a result, there is a high research interest in this good-tasting and highly traded percid species. The effects of different production systems on pikeperch growth and health are well studied (e. g. Zakes et al. 2012, Policar et al. 2016, Baekelandt et al. 2018). However, all of these studies do not address muscle tissue and its influence on fish meat quality.

Our previous studies revealed significant differences in physical meat quality and lipid composition, dependent on the animal origin and season (Komolka et al., 2020, Tönißen et al., 2022). Based on these findings, we further investigated the cellular and genetic differences of muscle tissue and the resulting meat quality in pikeperch. In the present study, we compared the biochemical, genetic, and histological characteristics of muscle tissue of wild and farmed marketable pikeperch.

Methods

Pikeperch from recirculating aquaculture system (RAS) were obtained from the Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries, Germany. The fish were fed a commercial diet (Coppens Supreme-10, 4.5 mm pellet size) with a feeding rate of 1.0% per body weight. Wild pikeperch aged two years were sampled from a fishery at Hohen Sprenz Lake in Mecklenburg-Vorpommern, Germany. They comprised the same age and sampling period as the RAS fish. White muscle tissue analysis included biochemical parameters (nucleic acid amount, protein content), enzyme activity of CK (EC 2.7.3.2), LDH (EC 1.1.1.27) and ICDH (EC 1.1.1.42), gene and protein expression (PAX3, MYF5, IGF1, IGF2 and MSTN). In addition, muscle fibre structure at the caudal penduncle (H&E and NADH diaphorase staining) and immunohistological staining of myogenic progenitor cells (Pax3+, Myf5+) was performed. Significant differences were determined with a two-tailed Student´s t-test ($q < 0.05$) and a general linear model using SAS software version 9.4 (Statistical Analysis institute Inc., USA).

Results

The white muscle of wild pikeperch had a significantly higher RNA and DNA content than the RAS pikeperch, but the protein content in RAS fish is 30% higher ($p < 0.01$). In contrast to the comparable enzymatic activity of ICDH, the CK was 1.4 times higher and the LDH was 1.8 times higher ($p < 0.0001$) in wild pikeperch. This allow us a first look at the metabolic cycle of the pikeperch.

Furthermore, we were able to gain insights into the muscle fibre structure of pikeperch. The muscle structure differed in the white, intermediate and red fibre, but was not influenced by the origin of the fish from wild or RAS facilities. The number of nuclei per fibre cross-section were higher in the white fibre area ($p = 0.027$), but the nucleic density is higher in red fibre ($p < 0.001$). RAS pikeperch indicated significantly higher values in the myonuclear domain (ratio nuclei/μm^2) of white and red fibre compared to the wild animals. Additionally, immunhistological staining of the myogenic precursors revealed that in wild pikeperch, the distribution of $Pax3^+$ and $Myf5^+$ nuclei was not significantly different between the fibre types. However, the RAS animals had a significant higher proportion on $Pax3^+$ nuclei (group*fibre; $p = 0.03$) and reduced number of $MYf5^+$ nuclei ($p = 0.02$) in the white fibres. Due to this shift, the proportion of $Myf5^+$ nuclei in the red fibre area was twice as high as in the white fibre area. These results were confirmed by protein expression analysis. In RAS pikeperch the protein expression of Pax3 and Myf5 was significantly higher ($p < 0.0001$ rsp. $p < 0.01$) than in wild fish. In contrast, the white muscle-specific gene expression was comparable in wild and RAS pikeperch.

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Discussion

Due to its positive growth characteristics and the excellent taste of its fillet, the pikeperch is a promising candidate for RAS. The present comparative study to wild pikeperch allows us to investigate the effects of RAS husbandry on muscle tissue. Knowledge of pikeperch muscle tissue is important for successful breeding, rearing and growth in aquaculture systems as well as for ensuring fish meat quality.

Many of the muscle parameters presented here have been studied for the first time in pikeperch. For example, the biochemical performance parameters have shown that the species-specific and relatively constant DNA content is influenced by the cultivation of the pikeperch. The use of biochemical markers to assess nutritional or health status has been studied in other fish species (e.g. Ferguson et al. 1986, Hussna et al. 2020), but not in pikeperch. Our enzymatic data presented show that biochemical markers could be used in pikeperch aquaculture. Together with the gene/protein expression data and muscle structure analysis, this leads to a better understanding of the muscle tissue of pikeperch.

In general, the influence of abiotic and biotic factors on muscle physiology in pikeperch has not yet been sufficiently investigated. Our data demonstrate that some of the parameters studied are influenced by environment or origin. Therefore, the multiple parameters of the pikeperch muscle and their patterns presented here provide a basis for further analysis. How these parameters can be maintained or improved for the desired fish meat quality in aquaculture will be subject of further research.

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MULTIVARIATE ANALYSIS FOR DETERMINATION OF FISH HEMATOLOGICAL AND PLASMA BIOCHEMICAL RESPONSES TO WATER CONDITIONS

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Introduction

Since conventional statistical techniques cannot analyze large numbers of interrelated variables, a statistical approach with the power to extract principal components from a multitude of data needs to be applied (Sun et al., 2011). Application of mathematical and statistical methods used in order to process large data sets in an optimal way is termed multivariate data analysis (Kurtanjek and Gajdoš Kljusurić, 2014). Multivariate tools (MVA) used for reduction of variables, for classification and grouping, are Factor Analysis (FA) and Principal Component Analysis (PCA). They can give an overview of complex multivariate data and reveal relations between observed samples, allowing detection of significant variables as well as outliers (Bro and Smilde, 2014). The aim of the study thus was to assess the value and applicability of MVA for hematological and plasma biochemical responses of fish over two seasons, using physicochemical water properties and heavy metals concentration of water as determinants of multiple fish stressors.

Materials and methods

Important parameters were extracted from physicochemical water properties, heavy metals from water, leukogram with PCV values, and plasma biochemistry from *Carassius gibelio* blood, all measured in previous studies (Topić Popović et al, 2015). For this work, four different MVA tools were used; three of them were Analysis of Data and one was a Modeling Data tool. Analysis of Data tools: Agglomerative Hierarchical Clustering (AHC), FA and PCA. Modeling Data tool: Partial Least Square Regression (PLSR). After extracting primary parameters, PLSR was used to investigate the possibility for direct and rapid prediction of expected hematological and plasma biochemistry responses based on the water quality. Water and fish were sampled during the operation of a municipal wastewater treatment plant (WWTP) in NE Croatia. Representative seasons: spring (S) and fall (F). Representative sampling sites: 1. polluted canal receiving the outflow of treated wastewater (TW), and 2. unpolluted natural creek (NC) in vicinity of the WWTP.

Results and discussion

According to AHC, concentration of heavy metals had the same grouping pattern as plasma biochemistry (Figure). Clustering allowed grouping of observed data sets based on similarity of the observed cases, i.e. based on the season and the site of sampling. Clustering regarding fish plasma biochemistry showed an increase of linkage distance (> 45) for fish caught in spring in TW vs. NC. The same trend, but with larger linkage distance (> 200) was noted when the observed variables in the clustering process were heavy metals. Cluster analysis grouped the data based on the similarity of cases while FA, as an explorative analysis, grouped similar variables into dimensions, but FA does not distinguish between independent and dependent variables. It reduced the information by reducing dimensions of observations thus eliminating variables which were not significant enough (COD, ammonium, nitrite and nitrate, Hg and Pb, monocytes and PCV, and ALP). PCA analysis was conducted to determine similarities and differences in the observed sets of data depending on sampling sites and seasons, on the reduced data, and confirmed the AHC results. It also demonstrated which parameters were dominant in which season and on which sampling site. Unlike in the AHC method, there were quantitative differences for the observed parameters. PLSR was used to investigate a possibility for direct and rapid prediction of expected hematological and plasma biochemistry responses to water quality. It was an additional confirmation for the soundness of conducted methods of multivariate analyses, as well as the applicability of the model for prediction of expected parameters in the blood of *Carassius gibelio* based on water pollution in different seasons. The MVA tools provided valuable information on fish physiological status and stress impact on blood biochemistry.

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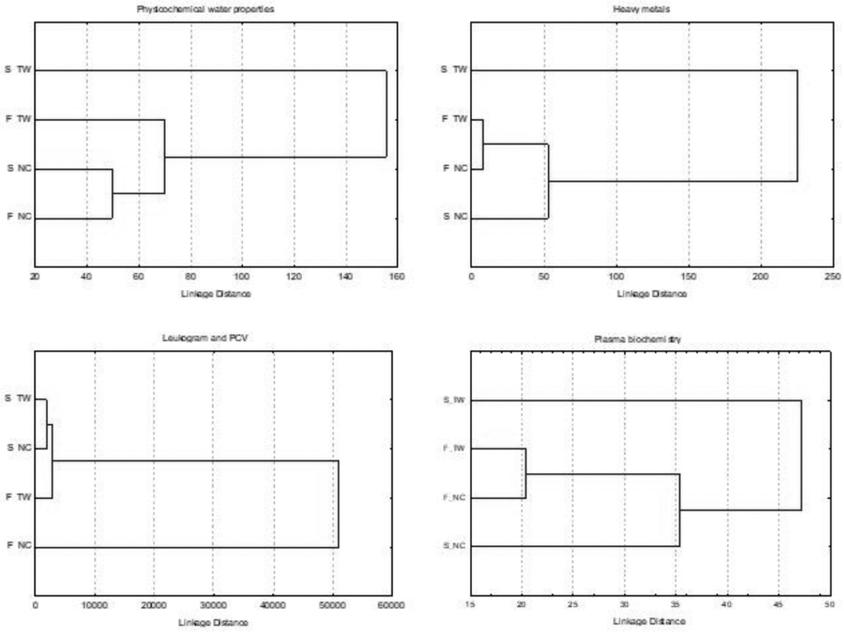

Figure: Agglomerative Hierarchical Clustering for physicochemical water properties, heavy metals in water, leukogram with PCV, and plasma biochemistry, containing data for two seasons (spring S/fall F) and two sampling sites (treated wastewater TW/natural creek NC).

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NUTRITIONAL INNOVATIONS IN SUPERIOR EUROPEAN SEA BASS (*Dicentrarchus labrax*) GENOTYPES: EFFECTS OF FUNCTIONAL FEEDS ON FISH PERFORMANCE, DISEASE RESISTANCE AND GUT HEALTH

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Introduction

A proper development of the aquaculture sector implies: (a) an effective replacement of marine raw ingredients (fish meal, FM; fish oil, FO) by emergent raw materials and (b) a successful breeding program addressed to improve growth, feed utilization and fish health. However, there is still some hesitancy towards the potential side effects of novel ingredients on critical aspects such as fish disease resistance, mucosal health or alterations on the fish gut microbiota homeostasis. Thus, to study if selected fish genotypes can benefit from the use of functional additives in aquafeeds to guarantee a future scenario covering the limitations on the availability of FM/FO, in terms of enhancing fish growth performance, mucosal health and disease resistance, is the aim of the present study.

Materials and methods

Two batches of European sea bass juveniles, high growth selected (HG; selected sires x selected dams) and wildtype (WT; wild sires x selected females) populations produced at MARBEC-IFREMER, were grown at the facilities of the Parque Científico-Tecnológico Marino (PCTM) at University of Las Palmas de Gran Canaria (Telde, Canary Islands, Spain). Fish were fed a "future diet", replacing totally FO by a combination of PO and DHA oil and reducing by a 50% FM content by PM inclusion (Skretting ARC, Norway), until achieving the initial experimental size of 16 g. After that, fish were randomly distributed in 24 tanks of 500 L (34 fish/tank; 12 tanks by genotype; 19.0 ± 0.4 g) and supplemented with 3 different experimental functional additives (INVE, Belgium) top-coated on future diet as follows: (i) 2 weeks at high dose followed by (ii) 10 weeks at low dose. The functional additives used were a probiotic mixture (PROB), organic acids mixture (ORG) and a phytogenic (PHYTO). Growth performance and feed utilization parameters were calculated for both feeding periods. Fish were sampled for gut integrity, functional microbiome and gut gene expression at the end of the total feeding trial. After each dosage feeding period, a pathogen challenge test (*Vibrio anguillarum*) plus stress condition (overcrowding) was applied to study the potential of additives to ameliorate the combined effect of both pathogen and stress condition. Fish were fed their respective diets along the challenge test and fish survival was recorded daily.

Results

No effects on weight gain were detected after 2 weeks of feeding in relation to fish genotype origin and functional diet supplemented. The results of the challenge test after two weeks are shown in Fig.1A (HG) and 1B (WT). A significant effect was detected for diet ($F=3.469$, $p=0.0411$), presenting fish fed the high dose of PROB a lower ($p<0.05$) survival than fish fed control diet, regardless of the fish genotype origin.

After 10 weeks of low dose experimental functional additives supplementation, HG fish presented a higher weight ($F=193.988$, $p<0.0001$) and SGR ($F=53.389$, $p<0.0001$) than WT fish, presenting fish the control diet higher ($p<0.05$) final weight than fish fed the PROB diet within the HG genotype group. WT fish presented lower total feed intake than HG fish at the end of the total feeding trial ($p<0.05$), regardless of the diet fed. After the final challenge test, diet fed influenced ($p<0.05$) the fish survival probability, presenting fish fed the ORG diet the lowest ($p<0.05$), irrespective of the fish genotype, whereas fish fed the future and PHYTO diets presented the lowest mortality in HG and WT groups, respectively (Fig.1C

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and 1D).In terms of GALT-related gene expression, HG fish presented lower ($p<0.05$) cyclooxygenase-2 (*cox-2*) gene expression that WT fish in both distal and proximal gut regions, regardless of the diet fed. The same effect was observed for cluster of differentiation 4 (*cd4*) gene expression in the proximal intestine. Neither the fish genotype nor diet affected the gene expression of *il-1b* gene, however fish fed PROB diet presented an upregulation ($p<0.05$) of *il-1b* gene expression compared to fish fed control diet within the HG genotype. Similarly, semiquantitative gut morphological analyses did not show evident changes in the incidence and patterns of distribution of intraepithelial lymphocytes, granulocytes, goblet cells, vacuoles and mucosal branching in both intestinal regions associated neither to diet nor fish genotype. Slight variations on the distribution pattern were detected for rodlet cells in fish fed functional diets compared to fish fed the future diet, regardless of the fish genotype origin. In terms of gut microbiota, discriminant analysis did not show a clear separation among fish fed the future diet and fish fed the experimental additives regardless of the genetic background, however variations on specific taxa relative abundance were detected. For example, fish fed ORG diet presented higher relative abundance of *Streptococcus* in both genotypes, whereas for fish fed PHYTO this effect was observed for Lactobacillales order and in fish fed PROB presented lower abundance of *Pseudomonas* and *Acinetobacter* genera.

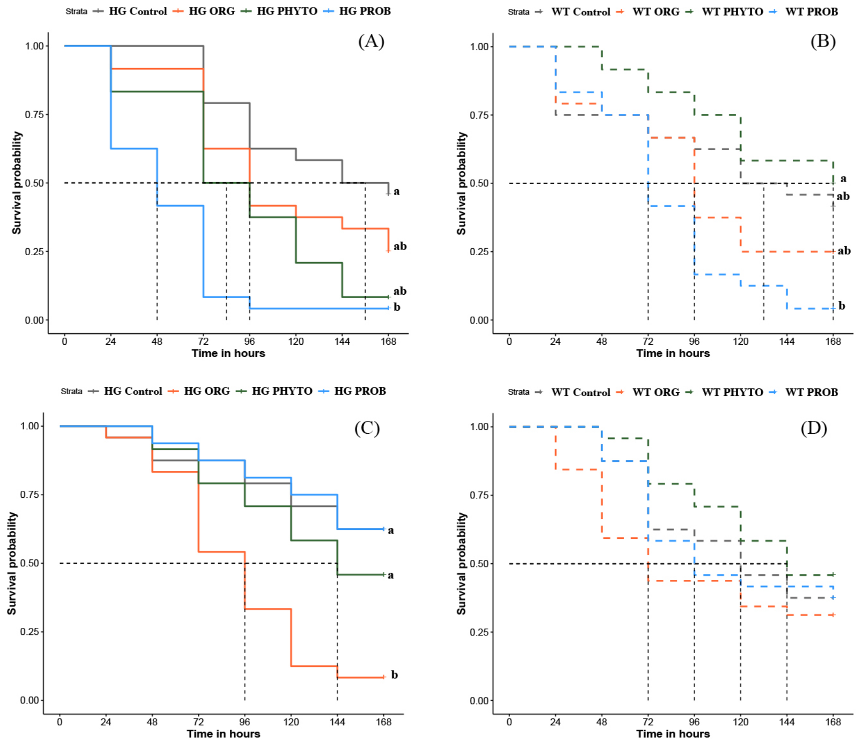

Fig. 1. Cumulative survival (%) of European sea bass (*Dicentrarchus labrax*) along the challenge test against *V. anguillarum* combined with stress by confinement after 2 weeks of high dose for HG (A) and for WT (B) genotypes and 12 weeks of feeding (2 weeks high dose + 10 weeks low dose) for HG (C) and for WT (D) genotypes. Different letters denote statistical differences ($p<0.05$; Kaplan-Meier survival). HG, genetically selected genotype; WT, wild type European sea bass genotype. Diets: probiotic mixture (PROB), organic acids mixture (ORG), phytogenic (PHYTO).

THE POTENTIAL FOR USE OF CAVIPLASMA DEVICE FOR ELIMINATION PATHOGENS IN WATER

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Currently, water contamination is of high importance and researchers are looking for new methods to purify water. Researchers from Brno (Czech Republic) developed a new technology for water purification using low-temperature plasma and cavitation. This device is called CaviPlasma which is now protected by a Czech patent (Rudolf et al., 2019). CaviPlasma can remove chemical residues such as oestrogens from contraceptive products and kill pathogenic microorganisms. First experiments with CaviPlasma demonstrated its exceptionally high efficacy in purifying water contaminated with cyanobacteria (*Microcystis aeruginosa*) (Maršálek et al., 2019). Another study demonstrated the ability of CaviPlasma-treated water to inactivate algae and cyanobacteria (Čech et al., 2020). We are considering the possibility of using this device to eliminate fish pathogens in water. We tested efficiency of CaviPlasma on the survival of bacteria (*Aeromonas salmonicida*, *Aeromonas hydrophila* and *Flavobacterium psychrophilum*). Thirty ml of bacterial suspension was inoculated into three l of tap water in sterile bottles and mixed well. 500 ml was used as positive control without treatment and 2,5 l was used for CaviPlasma treatment. Water with added bacteria underwent five passages through CaviPlasma device After each passage, samples of treated water were taken for futher analysis. During the first set of experiments the water flow rate was set to 1 m^3/h (ca. 17 slpm) and the separation of electrodes was approx. 16 cm. The backpressure was lowered to set the cavitation cloud spanning from the nozzle electrode to half the electrode distance. The discharge was energized using a HV generator set to approx. 65 kHz frequency at an input power of 600 W. The total treatment time of loaded batch of contaminated water was set to 65 seconds and 105 seconds. During the second set of experiments the separation of electrodes was set to 12 cm and the cavitation cloud spanned the entire inter-electrode space. At these conditions the input power was increased to 1 kW keeping the treatment times the same as previous. Samples of treated water were cultured at 17 °C and concentration of bacteria was regularly measured during the whole cultivation using plate count method. After cultivation, grown colonies were counted as colony forming units (CFU) per ml. In the first set of experiments, *Aeromonas* spp. survived in the treated water. The second set of experiments affected the survival of *Aeromonas* ssp. The bacteria were present in low quantities and gradually their quantities were decreasing. *Flavobacterium psychrophilum* was killed after only one passage of water in both sets.

We are testing the potential of CaviPlasma to eliminate a parasitic ciliate *Ichthyophthirius multifiliis* (the causative agent of the white spot disease). Water with developmental stages of *Ichthyophthirius multifiliis* was treated with CaviPlasma. This water underwent one or 5 passages through CaviPlasma device. Positive and negative control groups were included. The experiments on the effect of CaviPlasma on *Ichthyophthirius multifiliis* are in progress, the results will be presented as a poster at the conference.

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EFFECT OF TEMPERATURE ON DIGESTIVE ENZYME ACTIVITY OF EURASIAN PERCH (*Perca fluviatilis*)

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Introduction

Eurasian perch (*Perca fluviatilis*) is a fresh water carnivore that is relatively new in aquaculture and gaining increasing attention in recirculation aquaculture system in Europe. Eurasian perch culture is an expanding area of commercial fish farming in Europe. This species requires high protein diet formulated with marine fishmeal for balanced nutritional composition to optimize digestibility because of their very low feed consumption rate compared to other carnivore fishes. Several factors such as temperature, pH, diet, and age affect enzyme activity in the gastrointestinal tract of fish and thus can influence the biological availability of the feed. An alteration of the environmental temperature can affect food intake and gastrointestinal transit time, digestive enzyme activity, absorption of nutrients and how they store energy. There is a strong influence of temperature of fish due to their ectothermic nature. Knowing the activity of the digestive is important in designing feeding and rearing protocols which is suitable to the digestive physiology of respective fish species. The most important digestive enzyme in carnivore fishes are the protease enzymes (pepsin, trypsin, chymotrypsin and alkaline phosphatase activity) and lipase, although there is presence of amylase activity it is has less importance. The correlate of trypsin, chymotrypsin and alkaline phosphatase activity with fish growth has been established. The time it takes for food to pass through the gastrointestinal tract is influenced by numerous factors like fish species, size/age, food quality and quantity (ration and feeding frequency), and temperature. The aim of this study is to examine the digestive enzyme of Eurasian perch and determine which temperature is optimal for digestion.

Materials and Methods

For this study *Perca fluviatilis* (perch) fish (1-year-old, 50-60 g, 10 cm) were maintained at 16°C, 18°C, 20°C and 22°C, in 150 liters' aquarium and acclimatized for 48 hours and emptying of the digestive tract before feeding. The fish were fed commercial feed containing 48% protein and 13% fat content at first feeding 0,75% of their body mass and second time after 6 hours another 0,75% of their body mass. Fish were then euthanized 6.5 hours after the first feeding, then were dissected to gain their continuous digestive system (stomach, intestine) for further analyses. 25-25 specimens per treatment specimen were stored at -20°C until processing them into analytical samples. Enzyme activity measurements were carried out in three replicates per treatment by three aliquots. Pepsin activity was made according to Anson (1938) from gastric enzyme extract. Intestinal enzyme extract was used to measure trypsin activity, amylase activity, lipase activity, total alkaline protease activity according to Hummel (1959), Bernfeld (1955), Carriére et al. (1993), and Peña et al. (2015); García-Ortega et al. (2000) Walter (1984) respectively. The protein content of the enzyme extracts was used to correct the enzyme activities hence the results were given in Units/mg protein.

Result

The pepsin activity and trypsin activity at 18°C and 20°C is significantly higher than 16°C and 22°C. pepsin and trypsin activity follow the same trend, increasing from 16°C, peaking at 20°C and drastically reducing at 22°C There was significant difference in total alkaline proteolytic activity between 16°C and 22 °C but it was not significantly different at 18°C and 20°C. The lipase activity at 18°C was significantly higher than at 16°C, 20°C and 22°C. Although the lipase activity at 20°C and 22°C was lower than at 18°C, it was still significantly higher than at 16°C. The perch kept at 20°C and at 22°C had significantly higher α-amylase activity than those that were cultured at 16°C and at 18°C. Our result indicates that 18°C and 20°C seemed to be the most appropriate for maximal protease (pepsin, total alkaline proteolytic, trypsin) activity.

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Conclusion

Eurasian perch is carnivorous in feeding habit hence we recommend a temperature between 18°C -20°C for optimal protein and fat digestion, however it is important to consider other factors other than enzyme activity to find a balance when selecting an optimal rearing temperature. There have been recommendations of higher growth rate at 23°C, higher SGR at 26.5°C and easier control of infection at 20°C. Finding a balance between all this factor is the key to a successful production cycle.

EFFECTS OF HIGH STOCKING DENSITY AND MILD HYPOXIA ON GILTHEAD SEA BREAM INTESTINAL TRANSCRIPTOME AND MICROBIOME

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Introduction

Studies in gilthead sea bream evidenced that high stocking densities exacerbate the negative impact of reduced oxygen availability, showing transcriptional analyses a different contribution of analyzed tissues (liver ≥ heart > muscle > blood) to the hypoxic- and crowding stress-mediated responses to cope with a changing environment (Martos-Sitcha et al., 2019). Moreover, blood metabolic and muscle transcriptomic landmarks indicate that mild hypoxia induces a hypometabolic state, increasing the contribution of lipid metabolism to the whole energy supply to preserve the aerobic energy production during exercise (Naya-Català et al., 2021a). The intestinal microbiota also has key effects on host health and welfare, and these complex populations tightly interact with the host affecting local and systemic physiological functions (Naya-Català et al., 2021b). Thus, the aim of the current study was to assess how mild hypoxia and high stocking rearing conditions affect the intestinal health of gilthead sea bream by applying a hologenomic approach to determine the host-microbiota interactions.

Methods

Two-year-old gilthead sea bream (450-500 g) were tagged and distributed in 3,000 L tanks to achieve three different initial rearing densities (low, LD: 6 kg/m^3; medium, MD: 12 kg/m^3; high, HD: 22 kg/m^3). Fish were fed close to satiety with a commercial diet from May to June (8 weeks) under natural photoperiod and temperature conditions at our latitude (40°5’N;0°10’E), varying the concentration of dissolved oxygen from 6-5 ppm in LD fish to 5-4 ppm in MD fish, and 4-3 ppm in HD fish. At the end of the trial, the growth rate of HD fish was 40% lower than that of MD/LD fish, and randomly selected fish from each group were taken to obtain tissue and mucus samples from the anterior intestine. RNA extracted from the anterior intestine from 10 fish/group was sequenced using NovaSeq PE150 (50 M reads/sample), and reads were assembled and mapped against the gilthead sea bream genome. DESeq2 was used to extract genes with $P < 0.05$ in all comparisons, that were used to perform discriminant and cluster analysis to determine the number of differentially expressed genes among conditions. K-means, GO and KEGG enrichment analyses were subsequently performed. The V3-V4 regions of the 16S rRNA of the mucus DNA samples from the same animals were amplified and sequenced by Illumina MiSeq. After quality filtering, taxonomic assignment was performed with a custom-made pipeline using the RDP database. Alpha diversity was calculated using Phyloseq and beta diversity using PERMANOVA and PLS-DA models. Correlations between differentially expressed genes and discriminant bacteria were studied using the corrplot R package.

Results

RNAseq and discriminant analyses revealed that a total of 2,813 differentially expressed genes significantly separated the three groups (Fig. 1A). K-means analysis divided these genes into four different clusters according to their expression patterns in each group, separating one cluster of 800 genes with higher expression in LD, a second cluster of 1,103 genes with higher expression in MD, a third cluster of 688 genes with higher expression in HD, and a fourth cluster of 222 genes that gradually increased expression with the stocking density (LD<MD<HD). GO and KEGG enrichment analyses showed that genes with higher expression in LD were related to immune responses, including Th1 cell and tolerance-related pathways, inhibition of apoptosis and biogenesis. Genes with higher expression in HD were related to Th17 responses, hormone synthesis and circadian rhythms. The rearing density also affected gut microbiota composition. Although no changes in richness were detected, beta diversity index was clearly different among groups, being the HD group the most divergent. Thus, HD fish showed a significantly higher abundance of Actinobacteria, exemplified by the genus *Prauserella*. Conversely, MD and LD groups presented a higher abundance of Proteobacteria with

Conclusions

Crowding and mild hypoxia had a significant impact on gut health, evidenced by significant changes in host intestinal transcriptome and associated microbial population. These results offer the possibility of developing new tools and approaches for a more precise evaluation of welfare in farmed fish. The ultimate goal is to mitigate the negative impact of stressors related to intensive rearing and climate change on aquaculture production, promoting a more ethical and sustainable production.

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Reyranella as one of the most representative genera (Fig. 1B).

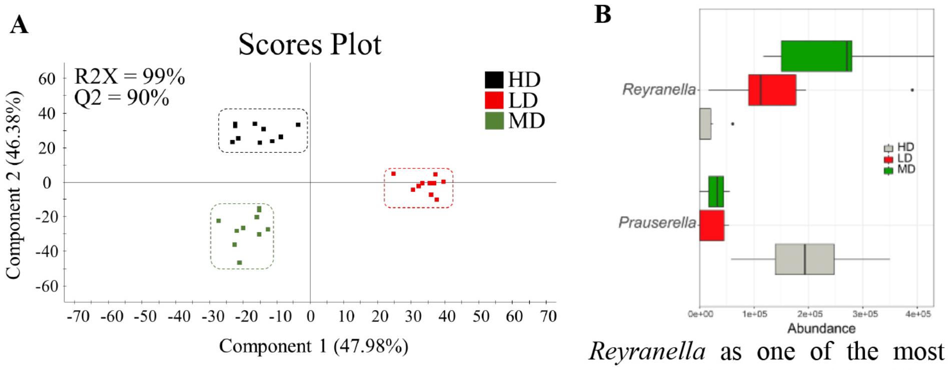

Figure 1: (A) Partial least-squares discriminant analysis (PLS-DA) showing group separation with the RNAseq data. (B) Linear discriminant analysis effect size (LEfSe) showing the two representative genera with more differences among groups.

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NANOPORE SEQUENCING REVEALS GROWTH RATE-DEPENDENT DIFFERENTIAL METHYLATION OF THE MITOGENOME IN NILE TILAPIA (*Oreochromis niloticus*) MUSCLE

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The mitochondrion is a cellular organelle that plays a central role in the regulation of respiration and metabolic processes in the cell, which are essential for muscle function and growth. Mitochondrial efficiency is directly linked with growth rate and varies among individuals, but the molecular basis of these differences is currently unknown. The aim of this study was to investigate a potential link between mtDNA methylation and growth rates in Nile tilapia (*Oreochromis niloticus*) muscle, since we have reported that the liver mitogenome is 20 % methylated (25 % CpGs) in this species. (Nedoluzhko et al., 2021). Fast muscle was sampled from slow and fast-growing Nile tilapia males and females, and used for genomic DNA isolation and construction of PCR-free libraries. In a representative library, the Nanopore MinION Mk1C sequencing workflow generated 673 Fast5 files, which were used for basecalling and methylation analysis with Megalodon v2.4.2 and Remora v0.1.2. The mean sequencing read length was 3275 bp and the coverage obtained for Nile tilapia mtDNA was 1082X. Methylation data generated from Nanopore long-read sequencing constitute a valuable resource for future research on the mitoepigenome related to muscle growth, metabolism and oxidation status of farmed fishes.

Acknowledgements

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INVESTIGATING CLIMATE CHANGE EFFECTS ON SCOTTISH SALMON AQUACULTURE & ASSESSING GENETIC MITIGATION – A PILOT STUDY

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Introduction

Rising seawater temperature as a result of climate change can have detrimental effects on aquaculture production, which is exposed to interactions with environmental factors. Seafood is the main source of animal protein for a billion people worldwide and has a key role in global food security, while such changes in production can have very negative socioeconomic impacts for local communities and national economies. Therefore, understanding the possible impacts of climate change on salmon production and exploring possibilities for mitigation, is crucial.

Previous studies in salmonids suggest that changes in seawater temperature can have negative impacts on fish health and welfare by causing stress (Anttila et al. 2013); for example, there has been observed between-family variation in thermal tolerance (Anttila et al. 2013), environment effects on sexual maturation (Wild et al. 1994), and temperature-related immune response changes associated with amoebic gill disease (Benedicenti et al. 2019). Research in salmon genetics has allowed to identify genomic variants that impact resistance to important infectious diseases (e.g. Houston et al. 2010) and growth (e.g. Tsai et al. 2015), and to optimise the use of genomic data for selective breeding in a cost-effective manner (Tsairidou et al. 2019). However, robustness to climate change effects has not been routinely included in breeding goals as yet, in part because this requires identifying suitable target traits. The aims of this pilot project were to (a) record and assess changes in major growth, survival, maturation and health traits in Atlantic salmon post-smolts, challenged with moderate and with more extreme heat-wave conditions on the west coast of Scotland; and, (b) investigate the existence of temperature-dependant genotype-by-environment interactions and the potential of selective breeding to improve temperature resilience as mitigation strategy.

Materials and Methods

This study focused on a Scottish Atlantic salmon breeding programme population of 518 salmon post-smolts, of age 13-14 months, from 54 families, which were challenged for 4 weeks in 3 tanks at (i) ambient temperature, (ii) ambient +4°C, and, (iii) ambient +8°C. Data were recorded pre- and after-treatment. Oxygen and water temperature were recorded daily reaching a maximum of 24.39°C, approximating heat-wave conditions for the west coast of Scotland. Fish were genotyped with the Hendrix Genetics multispecies SNP array (8,978 SNP genotypes for 492 fish after quality control).

Results and Discussion

There was a significant relationship between treatment and survival (p-value <5%), while ANCOVA analyses for growth traits including body weight, Fulton's performance index and average daily gain calculated for the duration of the trial, showed statistically significant differences between-tanks after treatment, indicating that a moderate increase of water temperature (+4°C) is beneficial for growth compared to ambient temperature, but further exceeding salmon's optimal zone (+8°C) has a negative impact on growth, possibly due to the additional energy requirements to cope with more severe heat-stress. Using ultrasound scanning, palpation and colour observation, it was observed that higher temperature treatments induced early maturation (grilsing), and an increase of AGD scores. QPCR essay for a range of pathogens and using 10 gill swab samples selected randomly (based on AGD scores) from each tank, revealed quantifiable Ct values for *Aeromonas hydrophila* and total bacterial load. *Aeromonas hydrophila* was detected on all swabs in higher temperatures, and is an opportunistic pathogen that can lead to outbreaks associated with increased water temperatures, mainly found in areas with warm climates. Structure analysis revealed the presence of three distinct clusters, hence principal components were fitted as covariates in all genetic models. Firstly, genomic heritability estimates were obtained across the entire population; growth traits provided moderate heritabilities, i.e. body weight and length before and after the trial (~0.39), and average daily gain (0.26). Binary survival estimated with both a linear and a generalised linear model, and day-of-death provided boundary small estimates (<0.05). Maturation (grilsing) provided a ~0.2 heritability, while for AGD score

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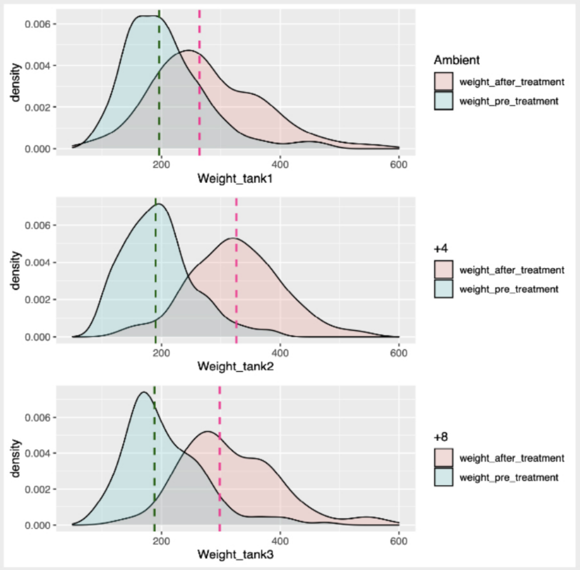

Figure 1. Distributions of body weight before and after treatment for each environment; dashed lines represent the median before (green) and after (pink) treatment.

it was not possible to detect genetic variance as the prevalence was extremely small. Within-tanks heritability estimates, showed small differences between-tanks, however, for growth traits heritabilities remained within the range observed in the overall population. For maturation the genetic variance that could be captured within each tank was very small with large standard error, while for survival traits it was possible to capture genetic variance only in the high temperature tank. This was reflected in the bivariate analyses to estimate covariances between environments; genetic correlations between-tanks for growth traits were very close to 1, indicating no significant re-ranking between environments, and for maturation the genetic correlations were very close to 1 but with large standard errors. Although in this data there was no indication for genotype-by-environment interactions, these results should be further validated in larger populations containing larger genetic variance in the traits of interest.

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DEVELOPING AN ANIMAL WELFARE ASSESSMENT AND CONTROL SYSTEM (AWACS) FOR FISH FARMS BY USING SOFTWARE THE SMART WAY

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Introduction

Within the globally growing aquaculture industry recirculating systems are getting more important. While these land-based systems have high-tech infrastructures, their potential for on-farm data management and analysis is far from being fully utilized. This hinders their operation on environmentally and economically optimal levels and lowers operational security and with it fish welfare.

Urban Blue offers land-based aquaculture farms a software solution which enables them to monitor key farm aspects and manage, analyse, and visualize this data to enable a better system operation. The Urban Blue system is a combination of a computer-based platform, hardware sensors and a mobile phone app allowing to assess the system (pumps, tanks, valves) and manage the operational workflow (task, routines, lists).

Together with the Zurich University of Applied Science Urban Blue has launched the Innosuissse innovation project AWACS (Animal Welfare Assessment and Control System for fish farms). The project aims at developing the automated assessment of fish-based parameters and adding them to the Urban Blue system in order to provide the aquaculture industry with a truly comprehensive solution, which allows fish-farms to constantly monitor, automatically assess and visually analyse fish health and welfare.

Safeguarding fish welfare by thinking worker-centred

Currently operational personnel on fish farms often reacts to imminent situations threatening the system's operation and the fish's welfare. Expanding data collection augments surveillance and available information about the system and fish. Improving data management enables personnel by moving from an experience-based to a knowledge-based farm operation. And enhancing data analysis facilitates early detection of problematic situations allowing workers to move away from reacting and towards acting. The key is to translate the farm's data into actionable insights and present them to the farm team in a useful way. To do this the AWACS team developed an innovative notification software based on flagged situations. This Urban Blue software assists data collection, management and analysis and with it empowers farm teams, secures system operation and safeguards fish health and welfare (Fig. 1).

Securing fish welfare by automating assessments

Currently operational personnel on fish farms often reacts to imminent situations threatening the system's operation and the fish's welfare. Expanding data collection augments surveillance and available information about the system and fish. Improving data management enables personnel by moving from an experience-based to a knowledge-based farm operation. And enhancing data analysis facilitates early detection of problematic situations allowing workers to move away from reacting and towards acting. The key is to translate the farm's data into actionable insights and present them to the farm team in a useful way. To do this the AWACS team developed an innovative notification software based on flagged situations. This Urban Blue software assists data collection, management and analysis and with it empowers farm teams, secures system operation and safeguards fish health and welfare (Fig. 1).

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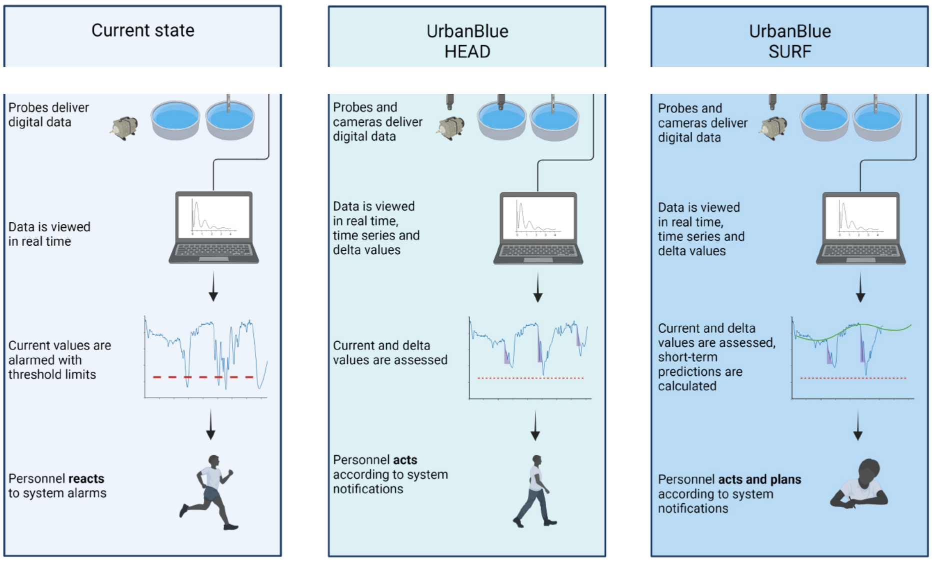

Figure 1: The Urban Blue products HEAD ad SURF with their positive impact on fish farm management and operational security.

Conclusion

Aquaculture can benefit greatly from the development of modern technologies such as data science and artificial intelligence. However, these methods will only be applicable in the industry if the focus is on the interface between farm workers and computer software.

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NEW INSIGHTS INTO GEOSMIN DYNAMICS IN FRESHWATER RECIRCULATING AQUACULTURE SYSTEMS FOR ATLANTIC SALMON *Salmo salar*

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Introduction

Aquaculture fish and shellfish production is continuously expanding due to increased demand and the simultaneous stagnation of capture fishery production. Due to the negative environmental impact of traditional intensive aquaculture, land-based fish and seafood production through recirculating aquaculture system (RAS) is considered as a great alternative. But despite the advantages of RAS (e.g. optimized water quality, reduced or no use of antibiotics, etc.) off-flavour substances (geosmin and methylisoborneol) can cause musty-earthy flavour in filets of fish which can lead to significant economic problems for RAS companies. Since off-flavour is not a critical factor for smolt production, which has a high technical level in freshwater RAS engineering, limited data is available about the formation and reduction of off-flavour substances in freshwater grow-out system. Therefore, many projects rely on purging processes.

Methods

Despite the common approach to improve the purge process, this study focuses on a reduction of geosmin in the rearing water, thus reducing the accumulation into the fish fillet. Understanding the origin and dynamics of geosmin production, accumulation and removal mechanisms in RAS is an important part of off-flavour management. By using a stable analysis procedure with GC-MS and high-resolution sampling, a commercial-sized RAS for Atlantic Salmon (*Salmo salar*) farming was monitored over 23 months. Dissolved and bound geosmin fractions were analysed in system water, sludge, biofilm on surfaces, biofilm on biochips and geosmin concentration in fish fillet.

Results

The results show a correlation between rearing water geosmin concentration and fish fillet. Concentrations in fillets are 50 to 100 times higher than in the rearing water. Rearing water geosmin concentrations can therefore be used to predict geosmin concentration in the fish fillets. Further on, mass balance equations considering input and output flows of the RAS show that the main removal mechanism for geosmin control was the solids removal unit. This leads to the conclusion that geosmin concentrations in RAS can be improved by solids control and do not mainly depend on the water exchange of the RAS.

Contrary to the widespread opinion that the RAS biofilter is the main/only source of off-flavour substances in RAS grow-out, we demonstrate that biofilm or deposits formed on various open sites within the RAS plant are a major contributor to off-flavour production and release. Thus, controlling the growth, disturbance and detachment of these unwanted biofilms plays a vital role in off-flavour management.

Conclusion

Understanding the dynamics of geosmin and other off-flavour substances in freshwater RAS is important for research, engineering, and operation. For research and operation, correct sampling strategies over long periods of time are important to understand geosmin dynamics. Regarding engineering and operation, preventing the growth and improving the removal of off-flavour producing microorganisms and the geosmin they produce profits from a deeper understanding of geosmin dynamics.

EFFECT OF DIETARY MANGANESE ON GROWTH AND SKELETAL DEVELOPMENT IN GILTHEAD SEABREAM (*Sparus aurata*) LARVAE

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Introduction

Manganese (Mn) is an essential micromineral involved in several biological processes such as protein metabolism, bone mineralization, immuno-modulator, and energy metabolism (Aschner and Aschner, 2005). Moreover, Mn plays an important role in antioxidant defence system, a Mn-dependent superoxide dismutase (manganese superoxide dismutase, Mn-SOD) which functions against oxidative damage in mitochondria. The deficiency of dietary Mn in juvenile fish caused poor mineralization, increased skeletal abnormalities, growth reduction, low Mn content in body tissue, and reduced liver Mn-SOD activity (Yamamoto et al., 1983, Antony Jesu Prabhu et al., 2016). In fact, the reduction of MnSOD caused by deficiency of Mn may alter the mitochondrial function resulting in mitochondrial dysfunction, ultimately increasing the production of Reactive Oxygen Species (ROS) and leading to oxidative stress (Li and Yang, 2018). The excessive ROS production may disrupt the balance between MSC (Mesenchymal stem cells)-mediated bone formation and HSC (Hematopoietic stem cells)-mediated bone resorption and thus leads to bone disease (Li et al., 2021). However, high Mn supplementation in diet (1000 mg/kg) reduce feed efficiency and body Fe, Ca, P concentration in grouper (Ye et al., 2009). Therefore, it is needed to define the optimum dietary Mn level to fulfil the Mn requirement level for fish.

Mn requirement studies have been carried out in several of juvenile fish (Antony Jesu Prabhu et al., 2016, Dominguez et al., 2020), the requirement levels vary from 2.4 – 24.9 mg/kg bases on the growth performance, vertebrae Mn and Liver Mn-SOD level. However, the effects of dietary Mn on growth, skeletal development and antioxidant activity have been scarcely studied in marine fish larvae. Copepods are part of the natural food for marine fish larvae, Mn content in copepods range between 8-25 mg/kg (Hamre et al., 2008)using copepods as feed. The present study compares the micronutrient concentrations in rotifers with those of copepods, with the aim of identifying nutrients that may be limiting for normal growth and development of cod larvae. An additional criterion used is the nutrient requirements given for fish in general, by NRC (1993. Hence, this study aimed to investigate the effect of dietary Mn level below 8 and beyond 25 mg/kg in growth, skeletal development, and antioxidant activity in gilthead seabream larvae. As, gilthead seabream larvae are prone to present skeletal anomalies during the early developmental stage (Boglione and Costa, 2011), this study would help in bridging the knowledge gap on Mn and its effect on skeletal development in gilthead seabream larvae.

Materials and methods

Diets. Six experimental microdiets based on squid meal and casein and containing different levels of Mn at the levels of 6.3, 39, 75, 87, 200 and 270 mg/kg were tested. Manganese sulfate monohydrate ($MnSO_4.H_2O$) was used as the Mn source. Larvae (dry weight: 0.29 ± 0.06 mg, total length 7.02 ± 0.71 mm, 23 dph) were randomly distributed into eighteen tanks (1800 individuals/ 200 L tank) and hand fed every 45 minutes from 8:00 am to 8:00 pm until 48 dph. Larvae dry weight and length were measured at different points: initial (23 dph), intermediates (29, 38, 43 dph) and final (48 dph). At the final sampling, larvae were collected for whole mount stain and gene expression, and the remaining larvae for mineral analysis. Daily mortality was calculated for survival rate. All data were tested for normality and homogeneity of variances and means compared by Tukey's test and p values were estimated using regression ($P < 0.05$). Quadratic and linear regressions were used to establish a relation between dietary Mn level and their effect on the different variables.

Results and Discussions

After 26 days of feeding, fish larvae fed increasing dietary Mn levels showed an increase in body weight and total length, followed by the linear regression (Fig.1 Left) and significantly ($P<0.05$) increased the Mn levels in larvae whole body content from 2.03 to 7.67 mg/kg (Fig.1Right). Noteworthy, in the previous study, Mn content at 7.5 mg/kg in the red seabream (*Pagrus major)* larvae fed with enriched artemia significantly increased the growth (Satoh et al., 2008). This suggesting the 7.67 mg Mn/kg in larvae whole body consumed from the diets may be one of the reasons causing an increase in the larval growth in the present study. This reflects the importance of Mn supplementation in the diet to maintain the normal growth in fish. Larvae survivals were not significantly affected by dietary Mn levels, average survival rate was 37%. The *mnsod* expression of larvae (48 dph) showed a significant ($P<0.05$) up regulation with increasing dietary Mn levels

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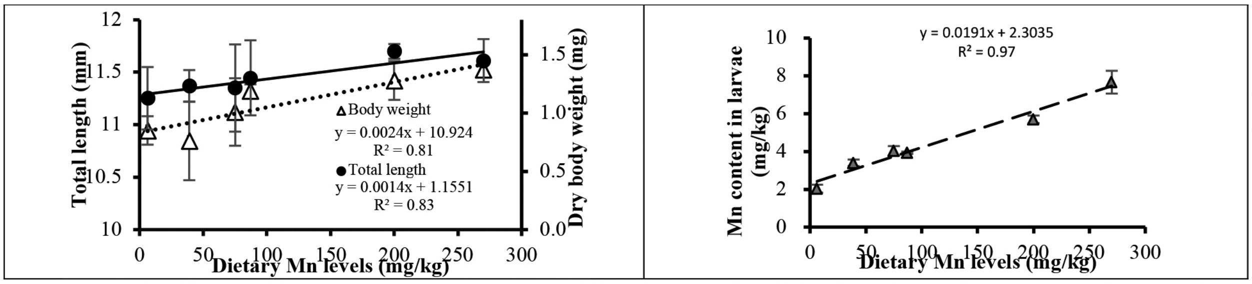

Figure 1: *Left.* Effect of dietary Mn on total length and body weight in gilthead seabream larvae (48 dph) following a linear regression ($p<0.05$). *Right.* Mn levels (mg/kg) in gilthead seabream larvae (48 dph) fed different diets after 26 days ($p<0.05$)

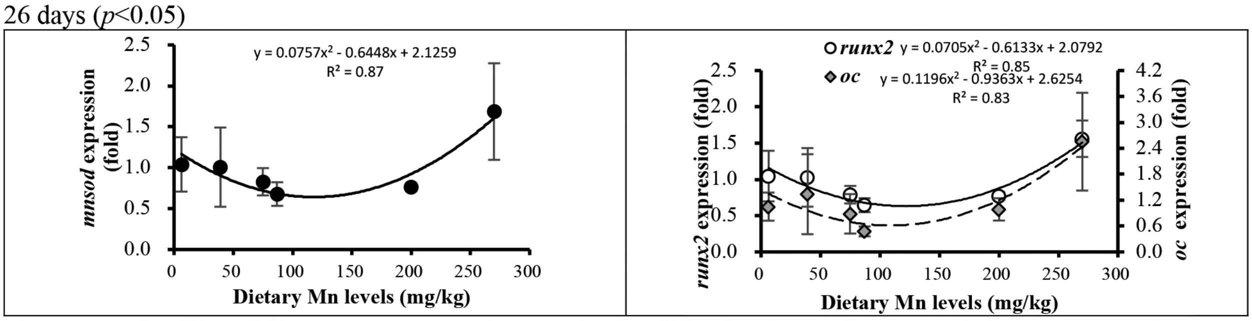

Figure 2: *Left.* Effect of dietary Mn on *mnsod* expression in gilthead seabream larvae (48 dph) ($p<0.05$) *Right.* Effect of dietary Mn on *runx2* and *oc* gene expression in gilthead seabream larvae (48 dph) fed different diets after 26 days ($p<0.05$).

(Fig.2 Left). A similar trend was found in the expression of *runx2* (Fig.2 Right), whereas *oc* genes showed the significantly ($P<0.05$) highest expression in larvae containing 7.67 mg Mn/kg fed the 270 mg Mn/kg diet thus resulted in a higher mineralization.

Conclusions

In summary, larvae fed the non-supplemented diet (6.3 mg Mn/kg) showed symptoms of Mn deficiency, including low growth, *oc* expression, Mn content in larvae and reduced bone mineralization. Whereas larvae fed with highest amount of dietary Mn level at 270 mg/kg didn't show signs of toxicity in gilthead seabream larvae at 48 dph.

Acknowledgment

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PARENTAGE ASSIGNMENT AND GENETIC PARAMETER ESTIMATION OF GROWTH-RELATED TRAITS AT DIFFERENT AGES IN MEAGRE *Argyrosomus regius*

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Introduction

Meagre (*Argyrosomus regius*) is a sciaenid species with a progressively growing scientific interest due to its great potential for aquaculture enhancement (Duncan *et al.* 2013). Towards this direction, in the present study, we attempt to assign offspring from a mass spawning event to their putative parents using a multiplex of 12 microsatellites and later combine the acquired pedigree information with phenotypic data of body weight and length at different timepoints, in order to estimate the genetic parameters of these economically important traits.

Material and Methods

Offspring coming from a hormone-induced spawning event of thirteen broodstock (of which 12 were injected with GnRHa) were reared together until grading at 297 Days Post Hatching (DPH), when big and small-sized fish were graded and transferred to different cages. At 394 DPH, 600 fish from each cage were randomly selected, individually tagged with a Passive Integrated Transporter (PIT-tag), fin-clipped and transferred into a rectangular cage. Weight was measured on all the surviving offspring at 394 DPH (BW1), 770 DPH (BW2) and 978 DPH (BW3). Additionally, length was measured on all surviving offspring at 770 and 978 DPH (Len2, Len3, respectively).

Fin clips sampled from all broodstock and offspring fish were used for DNA extractions. Genotypes were acquired using a multiplex of 12 microsatellite loci from Nousias *et al.* (2020, 2021) and later used for parentage analysis with the exclusion-based computational method, allowing for 2 mismatches. Heritability estimates as well as genetic and phenotypic correlations for the aforementioned traits (BW1, BW2, BW3, Len2, Len3) were obtained using a Restricted Maximum Likelihood method (REML), with a bivariate animal model in AIREMLF90 (Misztal *et al.* 2018) for all pairs of the studied phenotypes with the cage, in which fish were placed after grading, as a fixed effect.

Results

Parentage assignment and later genetic analysis included 1,179 offspring individuals, since 21 samples were excluded due to low DNA quality. The assignment rate of the parentage analysis was 71.8%, *i.e.*, 847 out of 1,179 offspring were successfully assigned to their putative parents with 0 to 2 mismatches. The 847 offspring were distributed in 24 out of the 42 expected families (6 males by 7 females), forming 3 large families (>15%) and 3 smaller families (5-9%). The rest of the formed families represented a very small percentage (< 2% each). All broodstock were identified as possible parents, except for one female. Unequal parental contribution was observed at both sexes.

Heritability for body weight and length ranged from 0.32 to 0.80 and 0.58 to 0.72, respectively, and tended to increase with age. Estimates were high for all the studied traits, except for body weight at 394 DPH which is within a medium range (0.20 – 0.40). Genetic correlation between body weight and length were high (>0.90) at both 770 and 978 DPH. Genetic correlation between body weight at different ages were lower (0.46-0.56). Finally, standard errors were quite high both for heritability and genetic correlation estimates. Most of the heritability and phenotypic correlation estimates exhibit statistical significance, while genetic correlation estimates were not significant.

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Discussion/Conclusion

Higher assignment rates (>90%) have already been reported in meagre in previous studies (Nousias *et al.* 2020; Vallecillos *et al.* 2021). The average assignment power of the multiplex used in this study can probably be attributed to high kinship within the broodstock as well as elevated inbreeding indices and low number of observed alleles (<6/locus) in the multiplex employed. The unbalanced family distribution and the consequent unequal parentage contribution has previously been reported in meagre (Nousias *et al.* 2020; Vallecillos *et al.* 2021) and other fish species (Liu *et al.* 2013).

The estimated heritability for body weight and length was relatively high and fell within the range reported by Nousias *et al.* (2020) but higher than those from Vallecillos *et al.* (2021), suggesting a possible highly profitable selection for these traits. The observed increase in the estimates of heritability with increasing age has been previously reported by Soula (2012) in meagre. The estimated values for genetic and phenotypic correaltions were very high between body weight and length at all studied ages, indicating the potential of using only one of these traits in selective breeding programs while also suggesting a possible pleiotropy and/or linkage among the genes affecting these traits. The respective genetic correlation estimates for body weight between different ages were lower. Last, the progressively smaller sample size due to high mortality in the studied offspring set could be the reason for the very high standard errors of the aforementioned estimates.

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DIEL OSCILLATIONS IN MICROBIAL PLANKTON COMMUNITIES IN AQUACULTURE ENVIRONMENT

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Introduction

Microbial plankton modulates oceanic function[1], is highly dynamic and oscillate over diurnal, seasonal and annual cycles. Diel oscillations are mainly driven by changes in light but also in nutrient and chlorophyll concentration, temperature and the presence of other organisms. The diel vertical migration of zooplankton has been studied in depth[2]. Prokaryotic community diversity, metabolism and abundance are frequently higher during the night[3,45,6], photosynthetic pigment production and cell division higher at night[7,8] while a consistent peak for heterotrophic protist and bacterial abundance is detected during the second half of the day[9]. The aim of this work is to describe diel oscillations in all microbial groups for the first time in aquaculture environment with metabarcoding analysis. We studied prokaryotic and eukaryotic diversity at 3 time points within the day and 3 sites having high, low and no impact from fish farming, and correlated diversity shifts with nutrients. The significance of nutrient pulses in shaping the dynamics of the basal members of all marine food webs is under investigation here, since it is possibly large enough to alter or “hijack” the microbial diel rhythms.

Material and Methods

Sampling took place within TAPAS (http://tapas-h2020.eu/) and LIFE+ AquaPEF projects (https://lifeaquapef.eu/) in areas where intensive aquaculture production of European seabass and gilthead seabream is occurring. Samples were collected from a site next to one fish cage (One Farm: OF) and another next to multiple fish cages (Multiple Farms: MF), and from a non-impacted site (Control: C), at 3 time points within the day [06:00 am (morning), 14:00 pm (noon), 22:00 pm (evening)]. Water was filtered for the collection of microbial plankton. Meso-plankton was collected via a vertical haul. DNA extraction was done following a CTAB protocol and PCR amplifications with a 2-step protocol[10]. The 16S rRNA gene was amplified for prokaryotes, the 18S for eukaryotes and the region ITS1-ITS2 for fungi. Sequencing was accomplished in the HCMR Illumina MiSeq platform. Sequence processing was done through PEMA v.2.1.4.[11] that operates in HCMR with high-performance computing cluster Zorbas. Reads of >150 nucleotides were organized into Operational Taxonomic Units (98% similarity) using Silva_132, PRS2 and MIDORI v2 databases. Differences in community composition were tested by applying multivariate analysis of variance using the factors “aquaculture” (Farm, Control), “type of aquaculture” (C, OF, MF) and “time of the day” (morning, noon, evening) using PRIMER v6 (Plymouth Marine Laboratory, UK). Distance-based redundancy analysis (dbRDA) was used to summarize the variation in the biological data that was explained by the abiotic data.

Results and Concluding remarks

Free and particle-attached prokaryotic communities were significantly different between C and Farm sites ($p<0.05$), while particle-attached prokaryotes differed also between morning, noon and evening. At both fractions, Gammaproteobacteria and Verrucomicrobia contributed more in Farm, while Cyanobacteria contributed more in C but showed substantially lower diversity. Interestingly, Chitinophagales, Cytophagales and *Rhodobacteraceae* increased from C to OF and further to MF, opposite to the SAR11 clade that decreased markedly from C to OF and further to MF. Meso-zooplankton community was significantly different between C, OF and MF ($p<0.05$), with the dominant phyla Arthropoda, Mollusca and Brachiopoda contributing more in Farm than C. Only some zooplankton phyla showed diurnal pattern. Diurnal effect was also detected for Dinoflagellata (decreased from morning to noon and further to evening), while Bacillariophyta and Radiolaria contributed less at Farm sites. Fungal community was significantly different between C and Farm and between C, OF and MF ($p<0.05$), although 65-85% of reads remained unassigned. All microbial communities were related to the concentrations of dissolved inorganic nitrogen forms and particulate organic carbon and nitrogen (dbRDA, $p<0.05$). Knowing the synthesis of a microbial plankton community may help us understand the function and performance of a given environment. This is

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particularly important in aquaculture areas that have major sustainability issues nowadays due to the substantial amounts of nutrients that are released on a daily basis. Our study showed that prokaryotic community was significantly affected by fish farming. Higher diversity, with taxa known for their opportunistic behavior (i.e. *Rhodobacteraceae*) prevailed near fish cages, while oligotrophic taxa increased in contribution away from cages. The eukaryotic community, including fungi, also differed with respect to farming, with diatoms contributing less near fish cages. The time of the day seemed to affect only some zooplankton phyla (but not the whole community) and particle-attached prokaryotes that are largely affected by organic matter produced by phytoplankton, exuded by zooplankton and released due to the farming operation. Altogether, aquaculture seemed to have a great effect in community synthesis which was supported by the consistent association with nitrogen forms, while the diel oscillations were milder and observed in specific taxa. Diel rhythms may be hijacked by continuous nutrient supplies within the aquaculture environment, altering ecosystem function, and thus, requiring further investigation for the consequences.

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DEVELOPMENT OF A MOLECULAR INDEX OF LARVAL QUALITY FOR USE IN COMMERCIAL PRODUCTION OF GILTHEAD SEABREAM AND EUROPEAN SEABASS

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Introduction

Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) are emblematic species of Mediterranean aquaculture, the second most important aquaculture industry after the Atlantic salmon in the European Union (Llorente et al. 2020). Hatchery stages are critical to the whole production cycle, as they determine the plasticity of body morphology and shape, growth potential and robustness (Kourkouta et al. 2021a, 2021b). Currently, larval quality is assessed through a series of Key Performance Indicators at different stages of development based on the desired traits of fast-growth, robustness of juveniles and adults free of morpho-anatomical defects (Kourkouta et al. 2021b). The aim of the present study was to screen for a set of molecular markers that could be used as quality indices for gilthead seabream and European sea bass larvae, and to develop a time- and cost-efficient tool for real-time batch quality evaluation in the hatchery.

Materials and methods

Gilthead sea bream (SBG) and European seabass (BSS) larvae of four stages [first feeding (FF), flexion (FL), end of larva rearing (ELR) and mid metamorphosis (MM)] were sampled from four and three commercial European hatcheries, respectively. Samples were used for RNA extraction followed by quantitative PCR (qPCR) and whole mount bone staining. For skeletal quality assessment alcian blue /alizarin red whole mount-stained larvae were analysed to establish the incidence of malformations. For both species quality was scored, based on the existence and the severity of deformities (Kourkouta et al. 2021a, 2021b). In the case of BSS, an additional quality score based on axial growth rate was used. Only, the best (BEST) and worst (POOR) performing production batches were used for further analysis. Following total RNA extraction and cDNA synthesis, the expression levels of 22 and 24 genes were measured by qPCR in SBG and BSS, respectively. A Wilcoxon signed-rank test was used to examine significant differences per gene and stage between the two quality extremes scored, BEST and POOR. The multi-collinearity of the variables in the data set, was assessed with a Pearson correlation coefficient. A multivariate approach and permutational analysis of variance (PERMANOVA) evaluated differences in gene expression and the size of the variability explained by the quality score. Random forest was trained as the classification algorithm using the gene profile obtained for the samples of known quality i.e. BEST and POOR. A quality prediction model was built and then used to classify samples for which the quality was masked before analysis.

Table 1. Accuracy, sensitivity and specificity of random forest algorithm for the examined stages and quality scorings for both SBG and BSS

Species	Stage	Accuracy	Sensitivity	Specificity	Quality scoring based on….
SBG	MM	**0.86**	0.90	0.83	Deformities
BSS	ELR	**0.86**	0.77	1.00	Deformities
BSS	MM	**0.84**	0.80	0.88	Deformities
BSS	ELR	**0.80**	0.93	0.60	Growth
BSS	MM	**0.88**	0.92	0.83	Growth

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Results and Discussion

The general strategy applied for both species was as follows: the first step was to screen for candidate genes that presented significant differences between the two quality scores (BEST vs POOR). The genes with no significant differences were excluded from the rest of the analysis. In the second step, correlation analysis was used to identify highly correlated genes (correlation coefficient of > 0.7) in each of the four stages. From each group of highly correlated genes, only the gene with the highest p-value in the Wilcoxon signed-rank test, between samples from the two extremes of quality were used in the next step of the analysis. Based on the criteria outlined above, sets of quality-related genes were selected for each stage. Subsequently, the PERMANOVA results were used to identify the stage/stages in which the differences between the two quality scores (BEST-POOR) were explained by the gene variables. Based on the results, genes were selected that were highly associated with quality at a given stage/stages for SBG and BSS and used for the random forest algorithm. The accuracy along with specificity and sensitivity of the applied random forest model for each stage and each species is presented in Table 1. Finally, the top five genes were identified that, when combined, contributed to generate a model predicting quality with high accuracy. A unique gene set was identified per species, stage, and quality criteria.

The study evaluated a set of genes that participate in key biological functions for their predictive power to estimate quality of commercial batches of SBG and BSS. This addresses a need for tools for real-time monitoring of batch quality during production cycles and may also reflect on the actual juvenile quality. The molecular index generated that consists of the top 5 most informative genes represents a novel, relatively fast and cost-effective tool for global larval quality screening. Finally, the accuracy of the model was ≥ 0.8 in all the cases. The algorithm could be further trained and refined for specific quality characteristics with the input of more data and by further validation with datasets for which quality has been masked.

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EFFECTS OF EARLY TEMPERATURE ON THE REGULATION OF OXPHOS COMPLEX I IN THE ONTOGENY OF GILHEAD SEABREAM (*Sparus aurata*)

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Introduction

In a constantly changing aquatic environment due to the climate change, the influence of temperature on fish physiology is becoming crucial for the future of fisheries and aquaculture. An increase in temperature leads to an increase in metabolic rates in most fish species, accompanied by increased respiration rates, and changes in survival rates, behavior, development and growth. Oxidative phosphorylation (OXPHOS) is a metabolic pathway that uses five multiprotein complexes localized inside the mitochondria to produce almost 90% of the cellular ATP needs in higher eukaryotes. In this study we investigated the impact of different early rearing temperatures on OXPHOS NADH: ubiquinone oxidoreductase (complex I) at distinct developmental stages in the gilthead seabream ontogeny.

Materials and methods

Gilthead seabream (*Sparus aurata*, SBG) eggs from a spontaneous spawn of captive breeders were subjected to 17, 20 and 23°C, from the onset of the epiboly to the end of yolk-sac larval stage and the beginning of exogenous feeding; subsequently all groups were kept under identical rearing conditions and constant rearing temperature (20°C) up to the end of the trial (18–19 mm TL). Larvae at three developmental stages, namely first feeding (FF), notochord flexion (FL) and mid metamorphosis (MM), were sampled and stored in methanol at -20 °C until RNA extraction. RNA was isolated from eight individual samples for each condition and were pooled. A typical pipeline for RNA sequencing was conducted on the pooled samples and the results were analyzed using the R package edgeR (Robinson MD et al., 2021). Most information about OXPHOS has derived from studies in human and other mammals. SBG genome was investigated for paralogs of CI genes in respect to the teleost specific genome duplication (TSGD), using the human peptide sequences as “bait” and following a reciprocal BLAST approach. All genes identified that code for complex I (CI) were included in the analysis.

Table 1 Influence of temperature to the number of differentially expressed genes per developmental stage

	17 - 20	**20 - 23**	**17 - 23**
FF	0	4	1
FL	3	0	0
MM	4	**10**	**29**

Table 2 Number of differentially expressed genes between the 3 developmental stages for the 3 rearing temperatures

	FF - FL	**FL - MM**	FF - MM
17°C	5	4	3
20°C	12	**28**	4
23°C	5	**33**	29

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Results and Discussion

Complex I is the largest of the five complexes of OXPHOS in terms of number of subunits, with 44 subunits in Human. From these subunits seven are coded from the mitochondrial genome and the rest from the nuclear genome. Fourteen genes code for the core subunits (7 mitochondrial coded and 7 nuclear coded) and they are responsible for the electron transfer through the enzyme and the proton pumping to the intermembrane space, while the rest, referred as supernumerary, contribute to the structure of the enzyme. From our analysis in the SBG genome we identified three CI nuclear-coded genes (*ndufv1*, *ndufs1* and *ndufs8*) that have retained paralogs compared to Human, and redefine the total number of CI genes in SBG to 47. RNA-seq analysis revealed a number of differentially expressed genes (DEG's) between developmental stages and rearing temperatures, with log fold changes ranging from 0.6 to 1.9 for numerus genes. It appears that the impact of early rearing temperature is low at FF and FL stages, whereas there is an increase to the number of DEGs at the MM stage for the larvae that had been exposed to 23°C (Table 1). Examining the changes to the number of DEGs at the transition between the developmental stages (Table 2), one can see that CI gene expression levels are stable between the developmental stages in larvae reared at 17°C with only a few exceptions, whereas a high number of DEGs were observed at the transition from FL to MM in larvae reared at 20°C and 23°C. Moreover, all the DEGs between FL and MM at 20°C and 23°C were up regulated at MM. Interestingly, one paralog from each of the three core gene families identified was highly expressed compared with the other in a consistent manner across all stages and temperatures of the study.

Oxidative phosphorylation is a crucial process for the cell and therefore, for the organism, and intense changes to the process or its regulation could result in low fitness or even death. On the other hand, the fine tuning of OXPHOS regulation provides plasticity that is important to cover efficiently the organismal energy needs under different circumstances. In this study, we provided evidence that different rearing temperatures, applied in a short time window at the beginning of development, can trigger changes in the regulation of OXPHOS CI later in development at the stage of MM, when the original thermal stimulus has been lifted, indicative of the epigenetic effects of early temperature. In conclusion, OXPHOS demonstrates, at least to some extent, thermal plasticity at the ontogeny of SBG. Further investigation is needed to fully understand the biological and physiological impact of the thermal plasticity of OXPHOS, in a way that can be manipulated for increasing SBG larvae and juvenile quality.

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HARVEST METHOD IMPACTS THE DEVELOPMENT OF EUROPEAN SEABASS SKIN MICROBIOME AT STORAGE

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Introduction

European seabass (*Dicentrarchus labrax*) is one of the most significant species farmed in the Mediterranean; yet a very perishable product. Its quality deteriorates rapidly as a result of three mechanisms: microbial activity, chemical oxidation, and enzymatic degradation. Microbial spoilage is the mechanism that contributes most to the quality deterioration of fresh and non-processed fish (Thomas et al., 2021)gills and intestines bacterial microbiome. Whole gilthead seabream was packed aerobically or under modified atmospheres (60% CO_2, 30% N_2, 10% O_2. The composition of initial microbiota is strongly dependent on many factors. The skin microbiome of unstressed fish is dominated by taxa recognized for their probiotic and antibacterial activities, whereas the skin microbiome of stressed fish is dominated by potential pathogens. And although mucosal surfaces, such as skin, gills, and the gut, operate as primary barriers to infections, they may be impacted by several pathogens. Stress can derive from anthropogenic stressors and farming conditions such as overcrowding and low oxygen concentrations and can cause variations in microbial composition, causing a shift in microbiome abundance (i.e., dysbiosis), leading to substantial losses. In addition, pathogenic bacteria that are generally prevalent in aquatic environments may also be present in fish microbiomes (Rosado et al., 2019)which is known to facilitate pathogen infections. The skin and gills are the primary defense organs against pathogens, thus, characterizing their microbiome composition in farmed fish is pivotal for detecting potential alterations that may lead to disease susceptibility. Here, we assessed the skin and gill microbiomes of two of the most important adult fish species farmed in southern Europe, the seabass and the seabream, during winter months. We coupled next-generation sequencing (MiSeq. Given the importance of the skin microbiome in fish innate immunity and the economic burden of fish losses in fish farming, characterization of the trajectory of the seabass skin microbiome is vital. To this end, our study aims to identify the composition and trajectory at storage of the European seabass skin microbiome from two distant aquaculture installations.

Materials and methods

Sampling of commercial size European sea bass (300-500g) took place at harvest days in two commercial fish farms in Western and Central Greece, at sea water temperature 20ºC and 21ºC, respectively. Fish were harvested using different methods; direct immersion in ice water or slurry ice; application of electrostunning prior to immersion in ice water. Samples were collected on day0 (harvest day) and day7 or day8 post-harvest using a sterile scalpel blade to swab several times along the right upper lateral part of the fish skin from head to tail in a sterile Eppendorf tube. Samples were kept at -80ºC until processing. DNA extraction was performed using the PureLink™ Genomic DNA Mini kit with modifications. DNA quality and quantity were assessed and then samples were pooled, properly diluted, and sent to BGI Genomics for 16S rDNA sequencing using the HiSeq 2500 Illumina platform. The selected area for sequencing was the hypervariable V3-V4 region of the 16S (small ribosomal subunit) gene. FASTQ files were analyzed with the DADA2 pipeline and clean sequences were aligned against the SILVA (138 release) reference database. All analyses were performed in R studio.

Results and Discussion

The analysis, after filtering for archaea, chloroplast, and mitochondria, showed that the 2571 amplicon sequence variants (ASVs) corresponded to 647 different genera. These genera belonged to 289 families. The genus present across all the comparisons was the genus *Psychrobacter.* Microbial composition revealed Proteobacteria and Bacteroidetes as the most abundant phyla, in accordance with the current knowledge on the skin microbiome of European seabass (Rosado et al., 2019)which is known to facilitate pathogen infections. The skin and gills are the primary defense organs against pathogens, thus, characterizing their microbiome composition in farmed fish is pivotal for detecting potential alterations that may lead to disease susceptibility. Here, we assessed the skin and gill microbiomes of two of the most important adult fish species farmed in southern Europe, the seabass and the seabream, during winter months. We coupled next-generation sequencing (MiSeq. When comparing the two fish farms only 135 ASVs, on harvest day, were shared with the farm in Western Greece revealing 680 unique ASVs of lower abundance (Figure 1a). The dominant genera in the latter farm were *Pseudomonas* and

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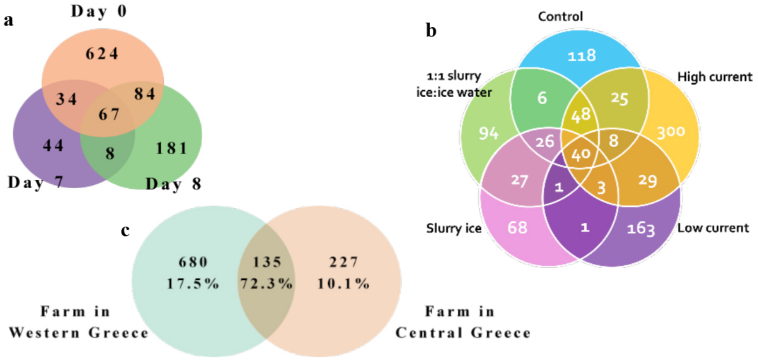

Figure 1a-c. Venn diagram showing the shared ASVs amongst the comparisons. The percentage shows the percentage of the sequences represented in the ASVs.

Pseudoalteromonas, whereas in the farm in Central Greece *Shewanella* and *Psychrobacter* genera were overrepresented (Figure1b). When comparing the different harvest methods used, only 40 ASVs were universally represented. The dominant genera for each harvest method were different, revealing the effect of the harvest method on the skin microbiome. Finally, when comparing the trajectories of the samples stored at 4ºC, only 67 ASVs were found to be shared by all samples (Figure 1c). By day7 or day8, the most prevalent genera were *Shewanella, Pseudomonas, Pseudoalteromonas,* and *Psychrobacter.* The relative abundance of each genus was strongly dependent on the harvest method. All these genera contain species such as *Shewanella putrefaciens, Pseudomonas aeruginosa, Pseudoalteromonas spp., and Psychrobacter sp.* that can be implicated in the deterioration and loss of the final product.

Conclusion

Differences in the microbiome composition of European seabass samples were observed, with an evident influence of the environment and harvesting procedures on the skin microbiome trajectory following storage at 4°C.

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TRANSCRIPTOME ANALYSIS OF OPSIN GENES - DELINEATION OF THEIR ONTOGENETIC PROFILE DURING EARLY DEVELOPMENTAL STAGES IN GILTHEAD SEABREAM (*Sparus aurata L.)*

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Introduction

Climate change is expected to impact all facets of primary production by driving developmental and physiological adaptations of farmed organisms to new trajectories. This is particularly important for poikilotherm organisms like fish that cannot regulate body temperature, with unforeseen ramifications for fisheries and aquaculture. Early developmental stages exhibit high thermal plasticity that can be decisive for robustness and growth performance at later stages. The purpose of this study is to explore how early thermal regime impacts in mechanisms associated with the perception of photoperiod in the gilthead seabream, *Sparus aurata L.*, an important species for the European aquaculture. Photoperception, is the ability of fish to perceive light, allows them to adapt their circadian rhythm, obtain an overview of their immediate environment in real time, detect their food, recognize predators, and seek potential partners for mating (2). Photoperception is mediated by optical cells, which are distinguished in rod and cone cells. Their outer parts contain photosensitive substances (pigments) that are different in rods and cones. All pigments contain an aldehyde of vitamin A and a protein called opsin. Opsin genes belong to G protein-coupled receptors (GPCRs) superfamily and form the core of vision. Their expression profile may be influenced by environmental factors such as water depth, water quality and tension, and they differentiated according to the wavelengths they can absorb. In this study we determined the ontogenetic profile of the opsins as it was shaped by different early thermal regimes as a means of information about fish photoreception (3).

Materials and methods

Gilthead seabream (SBG) eggs from a spontaneous spawn of captive breeders were subjected to 17, 20 and 23°C, from the onset of the epiboly to the end of yolk-sac larval stage and the beginning of exogenous feeding; subsequently all groups were kept under identical rearing conditions and constant rearing temperature (20°C) up to the end of the trial (18–19 mm TL). Larvae at three developmental stages, namely first feeding (FF), notochord flexion (FL) and mid metamorphosis (MM), were sampled and stored in methanol at -20 °C until RNA extraction. RNA was isolated from eight individual samples for each condition and were pooled. A typical pipeline for RNA sequencing was conducted on the pooled samples and the results were analyzed using the R package edgeR. SBG genome was investigated for paralogs of opsin genes in respect to the teleost specific genome duplication (TSGD), using the human peptide sequences as "bait" and following a reciprocal BLAST approach.

Results

In total, 37 opsin genes were identified in the SBG genome, 20 of which exhibited differential expression (p-adjust <0.05, absolute value of log fold change> 1) in pairwise comparisons between different stages, controlling for temperature. Of these, 5 belong to visual and the rest to non -visual opsins. Gene ontology enrichment analysis confirmed their involvement in photoperception mechanisms, absorption of visible light, cellular response to light stimulus and G protein-coupled receptor signalling pathway.

The expression of the paralog visual opsin genes followed reverse patterns; short wavelength sensitive opsin 1 (*sws1*), long wavelength sensitive opsin 1 (*lws1*) and the green wavelength sensitive opsin *(gws1)* were highly expressed at FF stage and their expression decreased thereafter. Conversely, an increased expression of *sw2* was recorded at mid metamorphosis, whereas its expression was low in the early stages. In addition, rhodopsin expression exhibited a gradual increase as development progressed. On the other hand, non-visual opsins expression gradually decreased as stages transitioned.

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Discussion and conclusion

To our knowledge this is the first study evaluating the expression patterns of opsins at different developmental stages in SBG. The biggest differences in expression were recorded between the FF and the FL stage. Increased expression of *sws1* and *lws1* genes at first feeding and gradual decrease at the other stages is viewed as directly related to the environment and depth of water, given juvenile fish inhabit euphotic water zones. The increase in *sws2* following metamorphosis coincides with their migration to deeper environments where the light spectrum is limited. Concerning rhodopsin, expression patterns appear to follow the morphological changes of fish during development, as the fish increases in size, so does gene expression (4). As for the non-visual opsins, they appear to be overexpressed at FF stage and their expression is reduced at later stages. These genes are involved in several basic mechanisms, such as induction and regulation of the circadian rhythm, and overexpression in early developmental stages is likely related to the adaptation to external stimuli and abrupt changes (5). The clarification of the effect of other factors, such as temperature, in the expression of circadian genes will provide us with useful information on the adaptive potential of fish and to address environmental challenges, such as climate change.

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RETHINKING PLASTIC PACKAGING FOR FISH AND SEAFOOD: NOVEL MATERIALS AND PROCESSES FOR A ZERO-WASTE FUTURE

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Introduction

According to FAO, significant amounts of fish and seafood are wasted worldwide due to spoilage and degradation during icing, packaging, storage, and transportation (ranging from 30-50%). . Developing novel and efficient packaging solutions to improve food quality and shelf life and reduce food waste while not raising the amount of packaging waste is a significant challenge for improving the sustainability of food supply chains. During the last decade, joint efforts by the packaging and the food industries have aimed to reduce the amount of petroleum-based polymers and food packaging waste (Tsironi and Taoukis, 2018). Biopolymers have been considered promising materials for this purpose. However, they generally present poor mechanical properties since the fragility and brittleness may limit their potential for industrial application. The properties of the developed materials depend on formulation and processing parameters. Edible packaging systems may be used as thin layers of materials to coat or wrap food products, aiming to extend their shelf life and reduce the required polymers for food preservation. In active packaging, the traditional role of packaging is accompanied by the conservation role of antimicrobials, antioxidants, and other components (Oreopoulou and Tsironi, 2021).

Polymeric surfaces are mainly hydrophobic, attributed to their characteristic low surface energy, and this implies that these do not possess the specific surface properties demanded in various applications. Moreover, the production of multi-layer structured food packaging polymers is economically demanding. To obtain polymers with the desired properties, in most instances various surface treatments are employed. Cold Atmospheric Plasma (CAP) induces several chemical and physical processes on the plasma-polymer interface, which modify the surface properties. These phenomena are at limited level exploited, to impart selective and tuneable surface energies to the packaging polymers for promoting adhesion or improved printability, sealability, imparting antimist properties and improving the polymer's resistance to mechanical failure (Hetemi and Pinson, 2017; Pankaj et al., 2014).

The objective of the study was to develop sustainable packaging materials by (a) the application of CAP for the surface modification of polymers, and (b) the partial replacement of plastics in packaging by the application of edible materials with high preservative effects for fish and seafood, including also the incorporation of active compounds with antimicrobial and/or antioxidant activity.

Materials and methods

CAP was applied directly on one side of a HDPE film and a LDPE film (20 μm and 40 μm thickness, respectively) using an atmospheric pressure cold plasma jet (kINPen® IND, neoplas GmbH, Germany) supplied with argon at a flow rate of 4 L/min and a conical probe of 16 cm diameter for uniform plasma application onto the film surface. Gas (O_2) permeability and water vapor transmission rate (WVTR) were evaluated using standardized methods (ASTM F2622, ASTM E96/E96M). Wettability of the packaging materials was characterized based on the determination of the contact angle (Theta Flow Optical Tensiometer, Biolin Scientific). Two alternative edible packaging systems were designed (a) an edible coating based on chitosan and acetic acid, and (b) an edible film based on carboxymethyl cellulose (CMC), sodium alginate (SA) and glycerol. The effectiveness and preservative effect of the developed packaging materials were evaluated on actual food environments (fish and seafood) through case studies and systematic shelf life testing.

Results

CAP increased OTR and decreased WVTR of PE films with the increase of treatment time. Wettability of PE films increased up to 48% after 30 min of CAP treatment, indicating a significant effect on the surface properties of both HDPE and LDPE.

The developed CMC-SA edible films had OTR and WVTR as 88218 cm^3*m^2/h and 14,514 g/m^2/h, respectively, indicating a high hydrophilicity in the developed edible system. A chitosan-acetic acid edible coating extended the shelf life of gilthead seabream fillets up to 5 days at 2°C, based on growth microbial spoilage (total viable count and *Pseudomonas* spp.)

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Discussion and conclusion

The results of the study show the potential of CAP treatment to modify the barrier properties of food packaging films. The developed methodology may be applied to a wide range of food contact surfaces, such as polypropylene and polyethylene-terephthalate, which alongside with PE account for more than 80% of food packaging polymers. The modification of packaging materials and the application of appropriate edible packaging will reduce petroleum-based plastics in a cost-effective manner with sustainable systems offering improved quality and extended shelf life of fish and seafood.

Acknowledgment

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SHELF LIFE EVALUATION OF FRESH FISH AND CRUSTACEANS USING NOVEL BIOPOLYMERS AS EDIBLE AND NON EDIBLE PACKAGING MATERIALS

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Introduction

Packaging of fish and seafood provides protection against chemical, biological, and physical modifications during storage. Recent research has been initiated to address three major trends in the food packaging sector, namely the health trend, the green movement, and the food safety trend (Tsironi and Taoukis, 2018). The recent trends towards the adoption of circular economy and the EU Directive 2019/904 regarding the reduction of the impact of plastic products on the environment has raised significant concerns about the sustainability and future of conventional polymers as food packaging materials. Polyvinyl alcohol (PVA) is a popular synthetic biodegradable polymer along with chitosan, a natural biopolymer obtained from crustaceans (Haghighi et al., 2019). Polylactic acid (PLA) is a biopolymer obtained from renewable resources, with similar properties to several conventional synthetic polymers (Ma and Wang, 2018; Armentano et al, 2015). Polyhydroxybutyrate (PHB) is an aliphatic polyester, synthesized by microorganisms, with high biodegradability and biocompatibility (Armentano et al, 2015). PLA/PHB blends have gained significant interest lately, since their combination can create new biomaterials with interesting physical, thermal, and mechanical properties (Ma and Wang, 2018; D'Anna et al., 2019). Biopolymers such as polysaccharides, proteins, and lipids may also replace petroleum-based plastics as alternative, green food packaging systems. Food-grade components can be used for the development of edible packaging materials. Pectin, gelatin and hydroxypropyl methylcellulose (HPMC) have been reported as effective raw materials for food packaging due to their non-toxic, biocompatible, and biodegradable properties. Pectin is a structural polysaccharide that comes from the cell walls of plants (Mellinas et al., 2020). Natural compounds with antimicrobial and/or antioxidant activity can be incorporated into active food packaging materials and delay spoilage of perishable foods, extending shelf life and reducing food waste (Oreopoulou and Tsironi, 2021). For example, eugenol is the basic compound of clove essential oil, "generally recognized as safe" (GRAS) and known for its antimicrobial and antioxidant activity (Li et al., 2021).

The objective of the study was to evaluate the shelf life of chilled gilthead seabream fillets and Pacific white shrimp, packed using alternative, environmentally friendly food packaging materials.

Materials and methods

Several alternative packaging films were developed by using a blend of PLA and PHB in a ratio 80/20 and glycerol monolaurate (GML) as a plasticizer (10% w/w of polymers), using the solvent casting method. Eugenol was added as an active compound in different concentrations (0, 0.1, 0.25, 0.5 and 1.0%v/v). PVA/chitosan films were also developed using GML as plasticizer. In order to synthesize edible films, pectin, gelatin and HPMC were used at a percentage of 2%, by solvent casting method. The solutions were mixed in proportions of 1:1, and three additional types of films were produced: pectin-gelatin, HPMC-pectin and HPMC-gelatin. The *in vitro* antimicrobial activity of active packaging materials was determined with a disk diffusion assay against *Pseudomonas fluorescens* (ATCC 13525) and *Escherichia coli* (UNIMORE 40522), while the *in vivo* antimicrobial activity was determined with the application of the developed films on fresh gilthead seabream (*Sparus aurata*) fillets or Pacific white shrimp (*Litopenaeus vannamei*) stored at refrigerated conditions (2°C). Shelf life evaluation was based on microbial spoilage (total viable count, *Pseudomonas* spp.), pH and sensory evaluation (appearance, odour).

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Results

The results showed that the edible materials developed by pectin-gelatin, HPMC-pectin and HPMC-gelatin may adequately replace petroleum-based polymers, such as polyvinyl chloride, and maintain a high quality of fish fillets without affecting shelf life. The antimicrobial activity of the PLA/PHB films against *P. fluorescens* was enhanced by increasing the concentration of eugenol, while eugenol was not effective against *E. coli*. At the end of the storage period of gilthead seabream fillets (i.e., 16 days at 2°C) the microbial load in the 0% eugenol films was 1 log cfu/g higher than the respective values for fish fillets packed in the 0.5% and 1% eugenol films.

Discussion and conclusion

The results of the study show the potential of PVA, PLA and PHB for the development of innovative, environmentally friendly packaging films for the preservation of fresh fish and crustaceans. Pectin, gelatin and HPMC edible films can be effectively applied as fish and seafood packaging systems for maintaining food quality and reducing the impact of packaging waste.

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COMPARISON STUDY OF USING TWO FEEDING REGIMES IN GILTHEAD SEA BREAM (*Sparus aurata*) UNDER DIFFERENT DISSOLVED OXYGEN SATURATION LEVELS

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Introduction

It is a fact that the way of feeding can affect the total amount of consumed feed, the fish growth and fish behaviour (Kentouri *et al.*,1994). Fish can control the feeding level according to their energy, nutritional requirements, and feeding time. An urgent need to develop a feeder to support aquaculture in cost effects and water pollution caused the thought of using a self-feeder system. The aim of this study was to compare the self-feeder feeding with hand-feeding in Seabream (*Sparus aurata)* under different oxygen saturation, investigating fish behaviour, growth performance and the quality of the final product.

Materials and Methods

Two different feeding regimes; automatic self-feeder system and feeding by hand, were implemented in Sea bream under two different ranges of dissolved oxygen (DO) saturation, 40-60% and 60-80%. The experiment was conducted in Aqualabs facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (Heraklion, Greece. Each diet was fed to three replicate groups (initial mean weight; 97.0±0.98 g, n=25) either by hand to apparent satiation twice per day or by automatic self-feeders (the fish ingested the food during the day) for 83 days. The trial was carried out in 3 closed recirculation systems (RAS) each consisting of 500L capacity tanks under fully controlled oxygen, light and water flow conditions. The rearing water temperature was 26.5°C and the photoperiod 12L:12D. The DO levels were selected based on results of previous trials and oxygen levels were fully audited and supported by an automatic oxygen system. The self-feeders used were connected to a special recorder where the frequency of activation during the 24 hours will be recorded. A camera system over each tank was placed to observe and record the fish's behaviour and activity. Software designed for this purpose informs their feeding frequency, consumption, and preference time.

Table 1. Growth / somatometric indices for Sea bream under two different feeding regimes and oxygen saturation levels.

Feeding regime	**Hand**	**Self-feeder**	**Hand**	**Self-feeder**
Dissolved oxygen %	**60-80**	**60-80**	**40-60**	**40-60**
Initial weight (g)	95.60 ± 0.44	97.00 ± 0.75	97.47 ± 0.40	97.77 ± 0.45
Final weight (g)	298.73 ± 0.94 ^{a}	300.42 ± 12.05 ^{a}	269.55 ± 10.21 ^{b}	266.58 ± 12.21 ^{b}
Daily growth rate - G (%)	2.56 ± 0.02 ^{a}	2.53 ± 0.17 ^{a}	2.13 ± 0.13 ^{b}	2.08 ± 0.14 ^{b}
Specific growth rate (SGR %)	1.37 ± 0.01 ^{a}	1.36 ± 0.06 ^{a}	1.23 ± 0.05 ^{b}	1.21 ± 0.05 ^{b}
Feed conversion ratio (FCR)	1.28 ± 0.02	1.34 ± 0.01	1.31 ± 0.12	1.21 ± 0.05
Daily feed consumption (DFC)	1.60 ± 0.03 ^{a}	1.65 ± 0.11 ^{a}	1.44 ± 0.09 ^{b}	1.32 ± 0.05 ^{b}
VSI	5.94 ± 1.08	6.82 ± 1.36	6.62 ± 0.85	5.98 ± 1.01
Perivisceral fat	1.86 ± 0.99	2.94 ± 1.05	2.50 ± 1.00	2.36 ± 0.63
HSI	1.02 ± 0.21	1.21 ± 0.19	1.07 ± 0.18	1.07 ± 0.25

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Results and Discussion

Growth indices for sea bream under two different feeding regimes and DO levels are shown in **Table 1**. Feeding regimes did not significantly affect final body weight, growth rates, FCR and daily feed consumption (P>0.05). However, the DO levels appeared to affect growth indices for sea bream, with better growth values presented by the groups of higher DO levels (60-80%, P<0.05). FCR was invariant between different feeding regimes and oxygen levels (P>0.05). In addition, neither the feeding regime nor the oxygen levels had any statistically significant effect on the somatometric indices for sea bream (P>0.05). The results of this study indicate that sea bream can achieve similar growth rates both by hand feeding and by using an automatic feeder by adjusting the amount of feed consumed per day. Feeding by hand proved to attain growth and feed consumption comparable to those fed by automatic self-feeders. In each case, the highest growth rates are achieved at the optimal oxygen levels for the species. The results of the present work regarding feeding regimes seem to coincide with those of Yamamoto et al. (2002) for juvenile rainbow trout (*Oncorhynchus mykiss*), where no differences in growth between hand and self-feeder feeding regimes occurred. Voluntary feed consumption and specific growth rates, as well as the hand-feeding amount, were significantly decreased when oxygen levels were low. Boujard and Médale (1994) observed that rainbow trout could regulate with great precision the amount of feed is consumed, regardless of the feeding technique. Hence, no differences in growth between hand and self-feeder feeding appeared. It has also been mentioned that continuous feeding (self-feeders) reduced the swimming activity and increased the aggressive fish encounters compared with the hand-feeding regime (Almazán-Rueda *et al*., 2004).

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CARP FARMING IN PONDS IN POLAND AS AN EXAMPLE OF AN ECOSYSTEM APPROACH IN AQUACULTURE

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Introduction

Aquaculture now provides half of the world's fish supply and is one of the fastest growing food production sectors in the world. Unfortunately, this growth is often accompanied by a number of negative phenomena, such as the occupation of large areas for aquaculture facilities, increased pollution, and the spread of disease. The integration of aquaculture into a broader system that takes into account the sustainable nature of socio-ecological interconnections is expected to prevent the above negative phenomena. Soto et al. (2008) found that three main principles underlie the development of environmentally and socially sustainable aquaculture: first, aquaculture must take into account the full range of ecosystem functions and services that should be provided to society in a non-threatening and sustainable manner; second, aquaculture should contribute to the well-being and equality of people representing all stakeholders; and third, aquaculture should be developed taking into account other sectors, policies and their objectives. Success in achieving the above goals requires, on the one hand, appropriate management of aquaculture development at the institutional level, and on the other hand, social acceptance of the measures taken. Pond carp farming is a type of aquaculture that is able to meet all of the above criteria.

Pond carp aquaculture in Poland

Poland has the largest area of carp ponds in the European Union, estimated at about 70,000 hectares. It is estimated that an additional 12,000-15,000 hectares is the total size of small rural ponds that are not distinguished in land records. Annual domestic carp production varies between 16,000 and 20,000 tons (Eurostat, 2022). Poland is the main European market for fresh carp, with a stable consumption of more than 21,000 mt. The carp aquaculture preserves its traditional character with low intensification of production (usually 450 – 500 kg/ha/year), and a large share of natural food (growing in the pond) in fish diet. Rearing common carp usually employs mixed species stock (polycultures). The share of other fish species, mainly grass carp, silver carp, crucian carp, tench, pikeperch, sturgeon, pike and trout, is around 13%. Low-intensive production, large areas of ponds and their open nature mean that the ponds, over decades and hundreds of years of use, have become an integral part of the local environment. They resemble vast, shallow lakes, providing a range of ecosystem services. The non-productive qualities of the ponds have helped expand the traditional carp economy to include tourism, recreation, catering, hospitality and other services (Békefi & Váradi, 2007). Pond farms that use traditional methods of fish production that are compatible with the needs of the environment and its protection can expect to be compensated for the additional costs incurred or income lost as a result. A multifunctional farm model that draws on tradition and culture has been identified as one of the primary strategic objectives for the development of domestic aquaculture (Figure 1).

Ecosystem services of carp ponds

The non-productive values of carp fish ponds have long been recognized by ecologists (Dobrowolski et al., 1994), fishing industry experts (Leopold, 1983) and fishermen (Turkowski, 2021). Studies conducted in France (Mathé & Rey-Valette, 2015) and Poland (Turkowski & Dubrowski, 2022) have shown that people not professionally involved in fishing are also fully aware of the importance of the environmental and social functions of carp ponds. This is the premise of public acceptance of the European fisheries policy, which aims to develop aquaculture without environmental deterioration, creating a balanced relationship between producers and the broader consumers of productive and non-productive aquaculture products.

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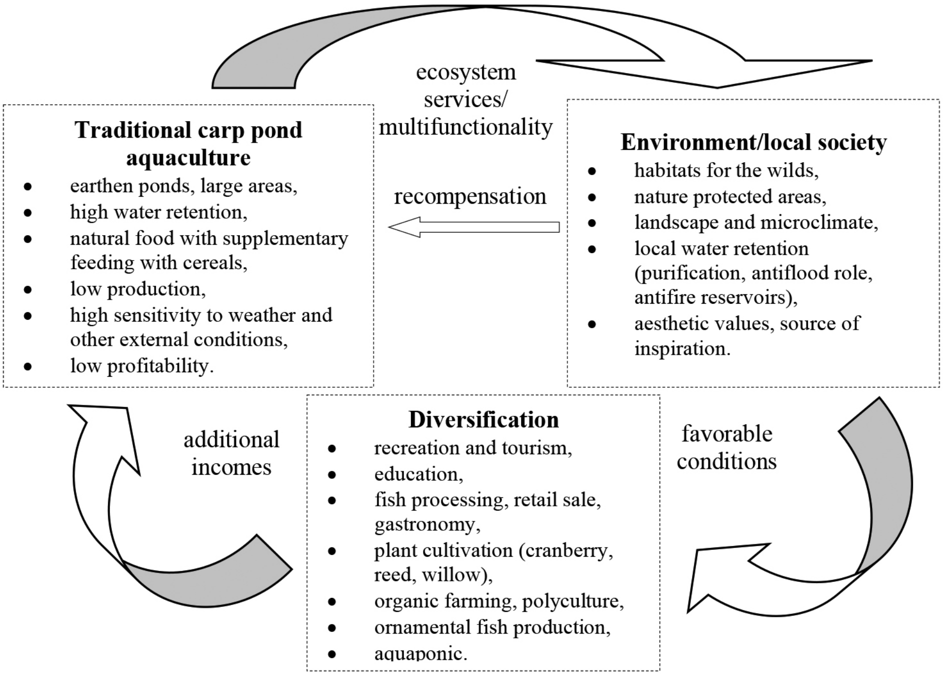

Fig. 1. Basic features of modern pond carp management. Source: Turkowski 2021, modified

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OFFSHORE SALMON AQUACULTURE FINANCIAL RISKS AND INVESTMENT ANALYSIS: IMPLICATIONS FOR GOVERNMENT REGULATIONS AND TAX REGIME

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Introduction

Marine aquaculture is today dominated by open cage inshore farming. Open cages can be very productive as they benefit from continuous inflow of huge volumes of water and discharge of effluents. But with increasing production and farm density open cage production may become less sustainable due to higher disease pressure and negative environmental effects in inshore waters. If it is not possible to increase inshore production using open cages in a sustainable manner, then society and industry can benefit from innovation in closed production systems or offshore farms that have smaller negative biological and disease externalities. Today we observe innovations and investments in these technological alternatives in Norwegian salmon aquaculture. However, the new offshore aquaculture sector faces significant biological, technical and financial risks. For government this may have significant implications for the design of regulations and tax regime.

The research questions we ask are as follows: (1) How do different investment costs and operating costs in offshore salmon aquaculture affect financial returns compared to conventional inshore salmon aquaculture? (2) How does the structure of biological, technological and financial risk differ between offshore and conventional inshore farming? (3) What are the implications for government offshore aquaculture regulations and tax regime to provide sufficient economic incentives for investment?

Empirical analysis

We provide a comparative empirical analysis of conventional salmon aquaculture and offshore aquaculture in Norway, which includes (1) an investment analysis of net financial returns (NPV) under different scenarios and employing sensitivity analysis, and (2) analysis decision trees which include decision points (e.g. investment decisions, government license allocation), uncertainties and risks for aquaculture investment projects. In the analysis we use firm-level economic data from the Norwegian Directorate of Fisheries, and data from companies on new offshore farm concepts.

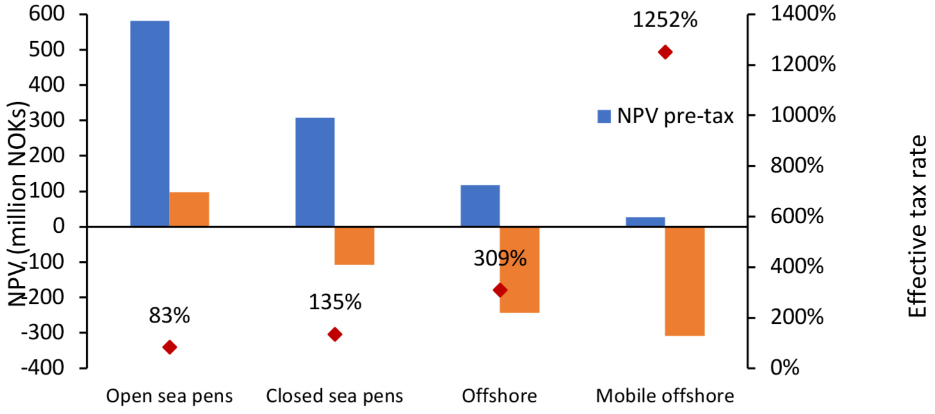

Figure 1. Net present value (NPV) before and after resource tax

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Offshore farms will have a much larger scale than conventional inshore farms: Typical production at inshore salmon farms is 2000-5000 metric tonnes (MT), while offshore farms may have a production volume of 10,000-25,000 MT. Investment in farm capital equipment (cages, feed barge, etc.) is typically 10-20 million Euro for a conventional inshore farm, while for an offshore farm investment costs may be 100-200 million Euro. Lead times from investment decisions to production start will also be considerably longer offshore, and with much higher negative cash flow before harvesting can begin. Offshore farming also represents a new production environment with generally stronger winds, higher waves, longer periods with weather that prohibits farm operations, and periods with extreme weather conditions. This implies higher biological and technical risk, and ultimately higher financial risks. It is reasonable to expect that increased risk compared to inshore farming should also lead to higher capital rate of return requirements for investors.

We calculate net present value (NPV) of aquaculture investment projects using different inshore and offshore cases and sensitivity analyses. The after-tax free cash flow at time *t* ($FCF_t^{before\ tax}$) is calculated as:

$$\mathrm{FCF}_t^{after\ tax} = (P_t \cdot Q_t - OPEX_t - DEP_t) \cdot (1 - \tau) - CAP_t - \Delta WC_t - IMM_t + DEP_t \tag{2}$$

where P_t is the salmon price (net of transportation and processing costs) at time *t*, Q_t is production volume, $OPEX_t$ is operation costs, DEP_t is depreciation, CAP_t is capital expenditures, ΔWC_t is change in working capital, IMM_t is investments in licenses (license fee to government), and τ is the tax rate on taxable income (in this case earnings before interest and taxes, i.e. EBIT). We also evaluate other tax structures.

We calculate the net present value (NPV) for different offshore and inshore projects, with different investment costs, operating costs, sales prices, rate of return requirements (discount rate), and taxes. As an example of the analyses we undertake, figure 1 shows the net present value before and after a resource tax, including effective tax rates.

Conclusions

Our analysis shows that offshore salmon farm concepts have much higher upfront investment costs and cumulative negative cash flows before the first harvesting, together with longer lead times until harvesting and significant new biological and technical risks. This should imply that investors' financial rate of return requirements will be higher in offshore aquaculture. Economies of scale and less negative biological externalities from inshore farms are potential benefits from offshore farming. We find that the NPV of offshore farm concepts are more sensitive to price and cost shocks than conventional inshore farms.

Our analysis also implies that the Norwegian government should not adopt all elements of the regulatory and tax regime currently present in inshore salmon aquaculture, but needs to consider innovations in regulations and taxation. This includes production license fees, tax rates, and timing of payments to government, which we analyze here. It also includes options to co-locate offshore farms in the same area to exploit economies of scale in farm production cycles, sea vessel logistics and energy infrastructure. With an appropriately designed regulatory and tax regime, government can provide incentives for investment in offshore farm projects that reduce biological and environmental externalities, and provide acceptable returns to society and industry.

EFFECT OF PARTIAL REPLACEMENT OF FISH MEAL WITH VARIEGATED GRASSHOPPER (*Zonocerus variegatus*) MEAL ON GROWTH, BODY COMPOSITION OF HYBRID AFRICAN CATFISH (*Clarias gariepinus X Heterobranchus bidorsalis*)

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Introduction

According to the FAO, Global fish production is estimated to have reached about 179 million tonnes in 2018, with a total estimated value of 250 billion dollars, of which 82 million tonnes valued at 250 billion dollars came from aquaculture production (FAO, 2020). Increasing aquaculture production has implications for the demand and price of aquaculture feed ingredients. There is an over-dependence by fish farmers on conventional feed ingredients such as Fish oil (FO), Fish meal (FM), soybean meal (SBM) as a source of protein, for which there is already an increase in demand because of the intensification of farming methods relying on complete fish feed (Tacon & Metian, 2008). FM, for example, is valued for its balanced amino acid and vitamin, which are essential nutrients in most fish feeds (Tacon, 2002). However, FM is the most expensive feed ingredient used in aquaculture feed formulation; due to increasing competition indirect human consumption. Edible grasshopper meal (GM) has been reported to have crude protein content between 29 to 61.50% (Alegbeleye et al., 2012; Okoye & Nnaji, 2005). Therefore, the study sought to evaluate the effect of partial replacement of FM with Variegated Grasshopper (*Zonocerus variegatus*) meal on growth, body composition of hybrid African catfish (*Clarias gariepinus x Heterobranchus bidorsalis*) which is one of the most cultured catfish species in Nigeria.

Methodology

90 days feeding trial was conducted to evaluate the effect of grasshopper (*Zonocerus variegatus*) meal (GM) inclusion in practical diets of hybrid African catfish (*Clarias gariepinus x Heterobranchus bidorsalis*). Five diets were formulated to include GM in hybrid African catfish diet at inclusion levels of 0, 10, 20, 30 and 40%; so as to partially replace 0% (control), 18.53%, 37.12%, 55.78% and 74.5% of respectively. The experimental diets were designated GM_0 (0% inclusion),GM_{10} (10% inclusion), GM_{20} (20% inclusion), GM_{30} (30% inclusion) and GM_{40} (40% inclusion). Hybrid African catfish fingerlings (n = 225) with a mean weight of 2.24±0.02 g were randomly distributed into 15 hapas with dimensions of 0.4m x 0.4m x 0.6m suspended in three concrete ponds and fed experimental diets in triplicate for 90 days.

Table 1: Growth performance and feed utilization of Hybrid catfish (*Clarias gariepinus x Heterobranchus bidorsalis*) fed increasing levels of GM in replacement of FM

	Control	**Treatments**			
	GM_0	**GM_{10}**	**GM_{20}**	**GM_{30}**	**GM_{40}**
Initial weight (g)	2.22 ± 0.00	2.22 ± 0.01	2.26 ± 0.02	2.24 ± 0.01	2.24 ± 0.01
Final weight (g)	31.45 ± 0.46^c	31.88 ± 0.16^c	33.33 ± 0.09^b	36.41 ± 0.14^a	31.53 ± 0.11^c
WG (%)	1304.70 ± 20.7^d	1336.04 ± 7.03^c	1374.81 ± 4.04^b	1525.54 ± 6.45^a	1296.21 ± 5.02^d
Survival (%)	100.0 ± 0.0	100.0 ± 0.0	92.0 ± 0.01	92.0 ± 0.0	92.0 ± 0.0
SGR (% day^{-1})	2.95 ± 0.02	2.96 ± 0.01	2.99 ± 0.01	3.10 ± 0.01	2.94 ± 0.55
FI (g)	36.72 ± 0.67^c	37.31 ± 0.42^b	37.93 ± 0.11^b	41.57 ± 0.34^a	36.86 ± 0.14^c
FE $^{1)}$	0.80 ± 0.01^b	0.80 ± 0.01^b	0.82 ± 0.01^a	0.82 ± 0.01^a	0.79 ±0.01^b
PER (g g^{-1})	2.14 ± 0.01^b	2.13 ± 0.01^b	2.19 ± 0.01^a	2.20 ± 0.01^a	2.13 ± 0.01^b
PR (%)	39.11 ± 0.08^b	38.98 ± 0.27^b	39.99 ± 0.16^a	40.05 ± 0.12^a	38.64 ± 0.22^b

Values are means ± standard deviation of triplicate samples. Means of different superscript within the same row are significantly different (P < .05). Abbreviations: WG: per cent weight gain; SGR: specific growth rate; FI: Feed intake; FE: feed efficiency; PER: Protein efficiency ratio; PR: Protein retention

(Continued on next page)

Results

At the end of the trial, no statistical differences between dietary treatments were observed for all haematological parameters and specific growth rate (SGR). However, statistical differences were observed for percentage weight gain (%WG), feed intake (FI), feed efficiency (FE), the protein efficiency ratio (PER), and protein retention (PR). Treatments fed GM_0 (control), and GM_{40} had significantly lower WG (%) and FI (g) than in GM_{10}, GM_{20}, and GM_{30}.

In comparison, treatment fed GM30 had significantly higher WG (%) and FI (g) than other treatments. In terms of FE, PER, and PR, treatments fed GM_{30} and GM_{20} had significantly higher values than other treatments. Treatments fed GM_{20} and GM_{30} had significantly higher VSI = Viscerosomatic index (VSI) value while those fed GM_{10} had the lowest VSI but did not significantly differ from those fed GM_0 and GM_{40}. Hepatosomatic index (HSI) was significantly lower in treatment fed GM_{10}, but no differences were detected in treatments fed the other inclusion levels. Whole-body composition showed no statistical differences among treatments for crude protein and ash content, but showed a difference for lipid and moisture content; lipid was significantly higher in the body of treatment fed GM_{40} while lipid was lowest in the body of treatment fed GM10 but did differ significantly from the treatment fed GM_0. Treatment fed GM_{40} had significantly lower moisture content in their body than treatments fed other dietary treatments. Protein ADC was significantly higher in treatments fed GM_{20}, and GM_{30}.

Discussion and Conclusion

Data from this study indicated that GM inclusion in dietary treatment significantly influenced growth, feed utilization parameter, hepatosomatic index, visceral somatic index, apparent digestibility coefficient, and whole-body composition of hybrid African catfish (*C. gariepinus x H. bidorsalis*); however, the hematological parameters of the fish were not influenced by GM inclusion. This study suggest that up to 55.82% of FM could be replaced by variegated grasshopper (*Z. variegatus*) without compromising the growth performance, survival, feed utilization, and health of hybrid African catfish (*C. gariepinus x H. bidorsalis*).

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EFFECT OF DIETARY VITAMIN D AND K ON SKELETAL DEVELOPMENT OF GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Skeletal development in hatchery reared gilthead seabream larvae (*Sparus aurata*) is a crucial process, where fish often present several anomalies such as lordosis, kyphosis, cranial malformations, and other vertebral anomalies (Izquierdo et al, 2019). This onset of skeletal anomalies lowers the survival rate by affecting the larval growth, swimming behaviour, and other physiological functions (Boglione and Costa, 2011). To overcome this issue, studies have tried to improve the larval production by altering the biotic and abiotic factors affecting larval skeletal development (Izquierdo et al., 2016). In accordance with that, nutrition is one of the major factors involved in skeletal development during early larval stages (Izquierdo et al. 2000). Hence, a series of trials focused on studying the effects of essential fat-soluble micronutrient vitamins D and K on gilthead seabream skeletal development during larval and juvenile stages were conducted. In addition, fish vertebra-derived cell lines were also used to understand the action of these vitamins on bone cell mineralization.

Vitamin D and K are essential micronutrients as they can't be synthesised *de novo* in fish (Lock et al., 2010, Krossoy 2011). They are involved in bone formation by regulating calcium metabolism and bone biomarkers in fish (Graff et al., 2016). They work synergistically to synthesise bone regulators such as osteocalcin (*oc*) and matrix Gla protein (*mgp*), which function in bone formation, mineralization, and soft tissue calcification, respectively (Graff et al., 2016). Deficiency of these vitamins in diet causes skeletal anomalies, growth reduction and increase mortality in fish (Udagawa 2000, Dominguez et al.,2021,2022). Thus, the aim of the study was to determine the dietary requirement of vitamin D and K and its physiological role on growth and skeletal development of gilthead seabream larvae, juvenile and skeletal derived-bone cells.

Experimental Design	Vitamin D	Vitamin K	Vitamin D & K Interaction
Larvae	✓	✓	✓
Juvenile	Dominguez et al., 2021	Dominguez et al., 2022	✓
Skeletal derived bone cells	✓	✓	✓

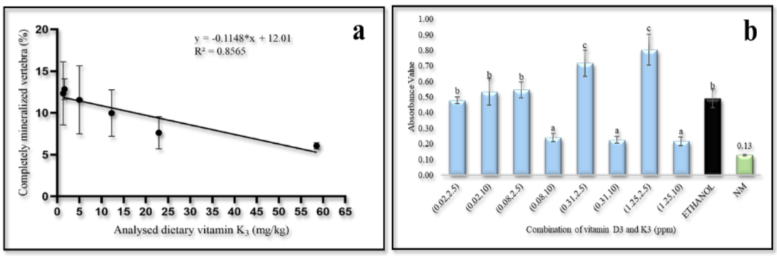

Figure 1: Effect of dietary (a) vitamin D_3 on percentage of completely mineralized vertebra in gilthead seabream larvae following a linear regression, $R^2 = 0.857$, $p = 0.008$ and (b) combination of vitamin D_3 and K_3 on extracellular matrix mineralization percentage in Vsa13 bone cells determined by one-way ANOVA ($p = 0.001$).

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Materials and methods

Four different *in vivo* experiments were conducted with dietary vitamin D and vitamin K, in which two were focused solely on vitamin D or K, while other two were aimed at understanding the interactions between both vitamins. Additionally, a similar model was designed for an *in vitro* cell culture study (Table 1).
Table 1. Experimental design and number of experiments conducted.

Gilthead seabream larvae and juvenile were obtained from Aquaculture research group (GIA), ECOAQUA Institute, University of las Palmas de Gran Canaria, Spain. For larval study, they were initially fed with enriched (ORIGREEN, Skretting, Norway) rotifer (*Brachinous plicatilis*) and randomly distributed into experimental tanks at a similar density. For the juvenile study, fish were screened for abnormal body shape, which were considered to present skeletal anomalies, were removed and the fish with normal body shape and a similar body weight were homogenously distributed in experimental tanks. At the end of the experimental period, fish were sampled for growth performance, skeletal anomalies, gene expression and other biochemical analysis. For the cell culture study, VSa13 bone derived cell lines from CCMAR (Centro de Ciências do Mar, Faro, Portugal) were used. The cells were regularly observed under the microscope and growth media (contains different dilution of vitamin) was changed at regular time intervals to understand the effect of vitamins in cells. Cultured cells were analysed for extracellular mineralisation (ECM), cell toxicity and gene expression.

Results and Discussion

Larvae fed increasing dietary vitamin D_3 showed an increase in complete vertebral mineralization with increase in severe skeletal anomalies and a reduced survival rate. The *bmp2* and *alp* gene expression were affected with the highest dietary group. Meanwhile larvae fed increasing dietary vitamin K_3 showed a reduction in complete vertebral mineralization (Figure 1a) with increasing abdominal kyphosis and reduced survival rate. Heatmap showed a reduced expression of osteocalcin (*oc*) gene expression with increase in dietary vitamin K_3. On the other hand, when larvae were fed with different combination of vitamin D and K there were no significant differences with skeletal anomalies among the groups and there was a tendency to reduce the larval survival at the group fed with the highest dietary vitamin D and K combination. The vitamin K-dependent proteins such as *oc* and *mgp* were highly expressed during day 7 and tended to reduce at day 21. The other bone biomarker gene such as *bmp2* were also affected on day 21 along with calcium regulators on both day 7 and day 21. However, when a different combination of dietary vitamin D and K were fed to gilthead seabream juvenile, no significant differences were observed among growth parameters and skeletal anomalies between the groups. Despite that, the combination of vitamin D and K upregulated the *bmp2* and *alp* gene expression and calcium regulators in gilthead seabream juveniles. In the case of*, in vitro* cell culture, the cells treated separately with vitamin D and vitamin K showed no significant difference in extra cellular mineralization (ECM). However, the combined effect of vitamin D and K showed significant reduction in ECM when treated with higher dilution of vitamin K with different vitamin D level (Figure 1 b).

Conclusion

- Analysed dietary level for gilthead seabream larvae between 25 - 30 μg/ kg of vitamin D_3, and 4.98 – 12.26 mg/ kg of vitamin K_3 would improve vertebral mineralization and reduce skeletal anomalies
- In gilthead seabream juveniles, the dietary combination of 0.04 mg/kg vitamin D_3 and 12 mg/kg vitamin K_3 diet would be recommended for optimum growth and reduced skeletal anomalies.
- The VSa13 *in vitro* cell culture suggested that an increase in the concentration of vitamin D_3 (0 – 2.5 ppm) and K_3 (0 – 20 ppm) and their interaction at (0.08-1.25 ppm VD and 10 ppm VK) caused cell toxicity and reduced extracellular matrix mineralization in bone cells.

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Acknowledgment

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EVALUATION AND COMPARISON OF USING UVA AND IRA IMAGING SYSTEMS TO OVERPASS THE LIMITATION OF WATER CLOUD ABSORBANCE

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Introduction

Water quality si an important attribute in many fields, including aquaculture production, biosystems protection and preservation. The water clarity could be affected by anorganic or organic contamination. The organic is represented mainly by the algae and cyanobacteria species, causing water blooms and increasing optical density. The anorganics are mainly minerals, soils, sand, mug, and clay particles. In case of fish welfare, biomonitoring, and bioimaging tasks depend on visible patterns of the fish body and behavior parameters. Therefore, it is crucial to achieve the adequate visual information, usually by digital camera and further processing and analysis of the obtained images or video. The turbidity affects the optical path differently for each wavelength of the light spectrum. The visible light suffers strongly with higher turbidity. In UV range, only the UVA is penetrating. The similar limitation is also valid for the imaging systems, namely CCD, CMOS, and Foveon detectors. The IRA part of the NIR spectrum is detectable by the visual cameras without the covering IR fiter.

Recently, the grow of the IoT and microcomputers allowed to to use pinhole cameras even as spectrophotometers. Therefore, optical properties of the water or water cloud could be also analyzed by the image processing and analysis. In this work we deals with the UVA and IRA imaging systems and investigate the possibility of such systems to overpass the turbidity effect, limitation, and dependency of absorbance caused by water cloud.

Materials and Methods

We achieved the measurement of visibility in part spectrum in artificial conditions in aquarium with different light sources (IRA, VIS, UVA). The digital image measurement itself was done in transmittance mode, the cameras were mounted on the opposite sides of the given light source, and therefore measuring the light shined through the water.

For the measurement we used the plastic chessboard like the Secchi disc, however instead of white there was blank, to be able to measure in transmittance mode. As a water cloud inducing substance was used the mineral clay. The additional measurement of the water samples was performed by portable spectrometer with standard plastic pathlend square cuvettes.

Results and Discusion

The absorbance mesurements were analysed using Vernier Spectral Analysis. The images were processed and evaluated by the MathWorks Matlab environment. the original RGB images were converted into grayscale intensity images, channels weighted respectively to each other. The optical visibility was evaluated via the root mean square contrast to compare the same water transparency properties for different imaging systems (IRA, VIS, UVA). The water turbidity could be from transparency point of view considered as a optical dence environment such as fog, where the IRA shows the best testing contrast ratio for direct beams. In practical use, the IRA imaging and capturing systém is used for evaluation in real conditions, like the monitoring of fish ladders in the rivers.

Acknowledgment

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WATER QUALITY IN *Cyprinid* FISH FARMS IN SERBIA, PRELIMINARY RESULTS OF A FIRST SURVEY IN THE FRAME OF DIRECTIVE 2006/44/EC

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Introduction

Fish farming in Serbia is mostly carried out in cyprinid and salmonid (trout) fish farms (over 95% of the total fish produced). Based on the official data from Statistical Office of the Republic of Serbia (2021) the total production of all fish species in cyprinid fish farms for human consumption in Serbia in 2020 was 4.761 t. The total number of cyprinid farms is 65. In Serbia, marketable carp (*Cyprinus carpio*) is often grown with other cyprinids (grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*) but carp fry is mostly grown in monoculture, often feed with the addition of supplemental diets. Water quality is the most important limiting factor in rearing of fish and directly affects feed efficiency, growth rate, fish health, and survival (Svobodova, 1993). It is therefore important to monitor water quality parameters to ensure they remain within the optimum range for farmed fish. As Serbia has become a candidate country for European Union membership, it is obliged to harmonize regulations in regard to water quality control in fish ponds, especially with Directive 2006/44/EC about the quality of fresh waters needing protection or improvement in order to support fish life (Directive, 2006).

To the best of the authors knowledge, in the past decades there no monitoring of water quality was carried out in cyprinid farms in Serbia The objective of this study was to screen the main physicochemical parameters of water in cyprinid ponds in different geographical areas of Serbia and compare them with recommended and mandatory values in the Directive 2006/44/EC.

Materials and Methods

Water quality was assessed in 12 ponds in total of 7 fish farms of cyprinid fish species. The geographical locations of investigated ponds in our study are shown on the map (Fig. 1). All samples were taken in October 2021. The total water surface area of the sampled ponds is calculated to be 2162

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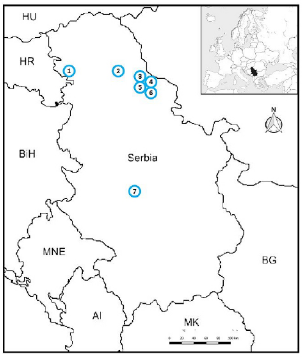
According to the rearing method, they belong to intensive (farms No. 1 and 7) and semi-intensive production (all other farms). Basic water quality parameters were measured on site: air and water temperature (thermometer, Testo, Germany), concentration of dissolved oxygen (DO), % of DO saturation and conductivity (oximeter and conductometer, Eutech Instruments, Netherlands), pH (pH meter, Testo, Germany), while portable photometer (Lovibond, Germany) was used to determine total ammonia (NH4+), unionized ammonium (NH3), carbonates (CaCO3), zinc (Zn) and free copper (Cu). Total suspended solids were determined by photometric method (photometer, Lovibond, Germany).

Figure 1. The map of fish farms in realized research survey



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Results and discussion

Water temperature at the time of sampling was within the recommended limit and ranged from 9°C to 13°C. Of the 12 ponds water samples tested, 4 (all in intensive system) and all 6 exceeded the recommended limits of ≤1mg/L and ≤0,025 mg/L of total ammonium and non-ionized ammonia, respectively. Of the 12 fishponds tested, 5 exceeded the recommended limits of 0.4 mg/L of total phosphorus. The levels of dissolved oxygen in accordance with percent of saturation were below desirable limits in 9 of the tested ponds: 50 % ≥ 8; 100 % ≥ 5. All of the tested ponds had higher concentration of dissolved copper than the recommended limits of ≤0,04 mg/L, while zinc was below detection limit. In this study, >70% of the ponds were found to be unfit for fish rearing based on their chemical parameters. The pH was in the range of 7,06 – 8,53, which belong to recommended values (6-9).

The findings of this study strongly suggest that there is big space for improvement of relatively poor water quality in selected fish ponds (e.g., high levels of copper). These results should help in the development and implementation of consistent national laws and regulations related to aquaculture production, based on the existing EU legislation and best practice currently implemented in EU Member States.

Acknowledgement

This research was funded by the French Ministry of European and Foreign Affairs in the frame of project “FAN-AQUA” granted in “ES BALK” - FSPI program (Fonds de Solidarité pour les Projets Innovants) and by the Ministry of Education, Science and Technological development of Serbia on the basis of the contract No. 451-03-68/2022-14/200117.

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PRELIMINARY RESULTS OF WATER QUALITY IN RAINBOW TROUT FARMS IN SERBIA - ARE WE CLOSER OR FURTHER FROM THE DIRECTIVE 2006/44/EC?

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Introduction

Fish farming in Serbia is mostly carried out in cyprinid and rainbow trout fish farms (over 95% of the total fish produced). Based on the official data from Statistical Office of the Republic of Serbia (Anonymous, 2021) the total production of rainbow trout fish farms for human consumption in Serbia in 2020 was 1.247 t. Whereas the total water surface used for rainbow trout (*Oncorhynchus mykiss*) breeding is known from official statistics (68,092 m^2), this is not the case with a number of rainbow trout farms. Because of difficulties to find an official source of this data, the total number of rainbow trout farms can only be estimated to be at least 100. In Serbia, marketable rainbow trout is only reared in open raceway systems with flow-through water circulation, especially in south - west regions of the country (located in highland regions). Traditional flow-through systems generally involve a multi-use (or multi-pass) of water, with different ponds supplied with flowing water. No water treatment is performed before being discharged to the receiving water, although some reuse might involve a supplementary oxygenation step. It is therefore important to monitor water quality parameters to ensure they remain within the optimum range for farmed fish. As Serbia has become a candidate country for European Union membership, it is obliged to harmonize regulations in regard to water quality control in fish ponds, especially with Directive 2006/44/EC about the quality of fresh waters needing protection or improvement in order to support fish life (Directive, 2006). The objective of this study was to screen the main physicochemical parameters of water in rainbow trout farms in different geographical areas of Serbia and to compare them with recommended and mandatory values in the Directive 2006/44/EC.

Materials and Methods

Water quality was assessed in 13 ponds in total of 4 rainbow trout fish farms of. The elevation of farms No. 1 and 2. was 477 m.a.s.l. and other two farms (3, 4) at 254 m.a.s.l. All samples were taken in March 2022. The number of investigated ponds per fish farm was 3 with exception of farm No. 4 (4 ponds). For each farm the measures were taken on three location: source of water (SW), ponds for juvenile stage (JS) and ponds of fish for consumption (FC). Basic water quality parameters were measured on site: air and water temperature (thermometer, Testo, Germany), concentration of dissolved oxygen (DO), % of DO saturation and conductivity (oximeter and conductometer, Eutech Instruments, Netherlands), pH (pH meter, Testo, Germany), while portable photometer (Lovibond, Germany) was used to determine total ammonia (NH4+), unionized ammonium (NH3), carbonates (CaCO3), zinc (Zn) and free copper (Cu). Total suspended solids were determined by photometric method (photometer, Lovibond, Germany).

Results and discussion

The results of our survey compared with recommended and mandatory values of Directive 2006/44/EC are shown in Table 1.

Water temperature at the time of sampling was within the recommended limits and ranged from 7,81 °C to 9,51 °C. Out of the 13 ponds water samples tested, the JS and FC of farm No. 1 and all three ponds of farm No. 2 exceeded the recommended limits of dissolved oxygen in accordance with percent of saturation (50 % $\geq$ 9 and 100 % $\geq$ 7). This has been a main problem in the past two decades because of a decrease in the water amount from the mountain rivers which supply these two farms. Concerning pH, it was in range of 7.43 – 8.40, which falls within recommended values (6-9), as well as zinc. Total ammonium and non-ionised ammonia were within mandatory range, $\leq$ 1mg/l NH_4^+ and $\leq$ 0.025 NH_3 respectively, but nitrites were slightly elevated, as well as total phosphorus and dissolved copper concentrations. The findings of this study suggest that there is a certain space for improvement of some parameters of water quality in selected fish ponds (e.g., high levels of copper). These results should help in the development and implementation of consistent national laws and regulations related to aquaculture production, based on the existing EU legislation and best practice currently implemented in EU Member States.

This research was funded by the French Ministry of European and Foreign Affairs in the frame of project “FAN-AQUA” granted in “ES BALK” - FSPI program (Fonds de Solidarité pour les Projets Innovants) and by the Ministry of Education, Science and Technological development of Serbia on the basis of the contract No. 451-03-68/2022-14/200117.

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Table 1. Main values of water quality parameters measured in 4 Serbian fish farms and reference values extracted from Directive 2006/44/EC for salmonid fish

Parameter	Units	Min.	Max.	Average	Guide values	Mandatory values
Water temperature	°C	7.8	12.5	9.5		21.5
pH		7.43	8.40	8.00		6 to 9
Suspended solids	mg/l	0.0	9.0	1.7	≤ 25	
Dissolved oxygen	mg/l O_2	5.8	14.5	11.0	50 % ≥ 9; 100 % ≥ 7	50 % ≥ 9
%O2	%	55	122	95		
Total ammonium	mg/l NH_4	0.903	0.516	0.297	≤ 0.04	≤ 1
Nitrites	mg/l NO_2	0.033	0.066	0.033	≤ 0.01	
Non-ionised ammonia	mg/l NH_3	0.000	0.021	0.003	≤ 0.005	≤ 0.025
Total P	mg/l PO_4	0.154	0.461	0.338		0.200
Water hardness	mg/l $CaCO_3$	106.00	192.00	149.85		10 to 500
Dissolved Cu	mg/l Cu	0.090	0.280	0.200	≤ 0.04	

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1. Statistical Office of the Republic of Serbia (2021), STAT database: Agriculture, Forestry and Fishery – Aquaculture. https://www.stat.gov.rs/en-US
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CYPRINID FISH FARMS IN THE HUNTING GROUNDS AND RAMSAR CONVENTION: EXAMPLE FROM NORTHERN SERBIA

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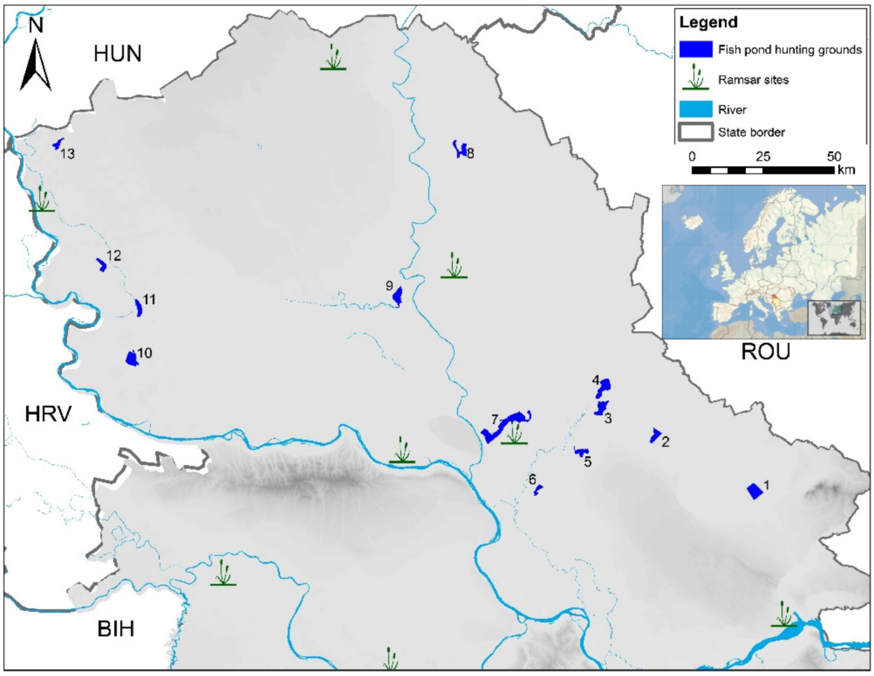
According to the Law on hunting (Official Gazette of RS, 18/2010) in Serbia hunting grounds are established as open or fenced. The open hunting ground cannot be less than 2,000 ha, except for special-purpose hunting grounds and hunting grounds on the surface of a registered fish pond. The hunting grounds on the surface of the registered fish ponds cannot be smaller than 200 ha. In the Autonomous Province Vojvodina (north part of Serbia) there are 13 hunting grounds of this type with a total area of 9501.89 ha. The hunting grounds on the map of the territory of northern Serbia (Province Vojvodina) are shown in Figure 1.

The main purpose of establishing these hunting grounds is primary for managing the population of great cormorants (*Phalacrocorax carbo*) and other species that present predatory for fish rearing in ponds. The great cormorant is a protected species according to the Serbian legislation (Ordinance, 2016), however, culling is permitted in the hunting grounds established around fisheries. According to the Proclamation on hunting seasons (Official Gazette of RS, 75/2016) great cormorants can be hunted during the entire year (1.1. - 31.12.). Hunting ground users may hunt exclusively to prevent damage in the fish ponds, following the annual hunting ground management plan. Besides preventing damages management plans also define cull quotas for the abundant species of waterfowls (ducks and geese) and other feathered game. These are important hunted species in the hunting tourism offer in the Vojvodina Region.

The Ramsar Convention (The Convention on Wetlands) refers to the conservation of wetlands of international importance, especially as waterfowl habitats. The Institute for Nature Conservation of Serbia has established a preliminary list of 68 potential Ramsar areas in Serbia.

Another important aspect of these hunting grounds is the fact that most of them are close to Ramsar sites, and present important habitats for endangered species. In Vojvodina, there are 8 Ramsar sites, which are shown on the map of the territory of northern Serbia (Figure 1). These 8 Ramsar sites cover a total area of 57.255 ha.

Figure 1. Map of northern Serbia showing the location of the surveyed fish farms



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While fish production is the main goal of registered fish ponds they also have great potential as generators of hunting tourism. Also, since wetlands are threatened these areas are valuable as habitats for birds. So, sustainable use of these resources could generate more funds that could be used for environmental protection.

All of these farms belong to the cyprinid farms, and rear firstly marketable carp (*Cyprinus carpio*) often together with other species grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) in the polyculture system.

The total water surface area of the showed farms is calculated to be 8.913 ha, which is 75 % of all cyprinid farms in Province Vojvodina, 6.681 ha in total. The water surface of the fish farms in hunting grounds ranged from 260 to 1.100 ha, in average 607 ha (Fig. 1). Most of them belong to semi-intensive system of fish production.

We can conclude that hunting tourism in most of a.m. areas is relatively developed but in some of them is still in its early phase according to the achieved turnover and tourism infrastructure. Contrary big potential exists in connection with other types of tourism (i.e. fishing) with connected offer of a.m. fish ponds in hunting areas and Ramsar sites in their neighborhood. Primarily because tourism activities are similar in character and potential. Directing economic development towards tourism could divert local communities from the activities which are traditionally present in the area but may significantly degrade it.

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 2022 (The Contract No. 451-03-68/2022-14/200117.).

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THE USE OF SUSTAINABLE MARINE LIPID SOURCES IN DIETS FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*): EFFECTS ON GROWTH PERFORMANCE AND FATTY ACID PROFILE

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Introduction

Aquaculture contributes for nearly half of the global food fish consumption, being a major source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for the burgeoning human population. However, as most marine fish species have limited ability to convert 18C fatty acids (FA) into long chain polyunsaturated fatty acids (LC-PUFA), fish farming is itself dependent on the dietary supply of those LC-PUFA, traditionally obtained from fish oil (FO) and fish meal (FM) (Tocher et al., 2019). In order to decrease the use of FM and FO in the aquafeed industry, it is necessary to find new sources of EPA and DHA in order to ensure adequate, sustainable and secure supply to the sector (Naylor et al., 2021).

The potential of marine alternative lipid sources (salmon oil, algae oil and a blend of micro and macroalgae) currently available in the market was compared to the traditional sardine oil used in aquafeeds. Sensible combinations were formulated to provide diets able to meet EPA and DHA dietary requirements of European sea bass and provide the consumer some differentiated fillet-rich in LC-PUFA.

Materials and methods

Six isolipidic diets (**Table 1**) were formulated targeting differential FA profiles. A control commercial-based diet (CTRL) was formulated with sardine and rapeseed oil as main lipid sources, with expected EPA+DHA levels of 2%. A positive control (SARDINE), targeting 3% EPA+DHA levels, included 8.5% sardine oil and 4.5% rapeseed oil. Three other experimental diets, were formulated to totally replace sardine oil by alternative sources rich in LC-PUFA, also targeting 3% of EPA+DHA: ALGASALMON with 9.9% salmon oil and 3.1% algae oil; ALGARAPE and ALGASOY with 4.4% algae oil and 8.6% rapeseed or soybean oil, respectively. A sixth diet was formulated to include 7.9% sardine oil, 4.7% rapeseed oil and a 2% blend of micro and macroalgae, also targeting 3% of EPA+DHA.

European sea bass individuals (initial body weight of 118g) were fed the experimental diets for 54 days. The trial was carried out in a recirculation aquaculture system (RAS) with water temperature at 20 °C. By the end of the trial, all fish were individually weighed to determine growth performance and feed efficiency. Fish were sampled for evaluation of nutrient utilization, whole-body and flesh FA content. Lipid quality indexes estimated from FA profile, concerning possible effects on human health, were also evaluated.

Results

All diets were equally well-accepted by the fish, no differences being found on feed efficiency or growth performance. A linear relationship between FA profile in tissues and in the diet suggested that EPA and DHA are retained in the muscle.

Although no differences were found in muscle total lipid content, the FA profile of sea bass fillet generally reflected the FA profile of diets. The muscle of fish fed ALGASALMON presented the highest PUFA n-3/n-6 ratio and ALGARAPE and ALGASOY the lowest, mostly due to the high linoleic acid content. EPA and DHA levels in muscle are presented in **Figure 1**. Muscle from fish fed ALGARAPE and ALGASOY had significantly higher DHA contents (231 and 218 mg $100g^{-1}$, respectively) when compared to CTRL, SARDINE and ALGABLEND diets. At end of the trial, all dietary treatments resulted in muscle EPA+DHA values that were within the recommended levels for human consumption to decrease the risk of cardiovascular diseases (> 250 mg $100g^{-1}$). A trend for ALGARAPE and ALGASOY to be more effective in increasing muscle EPA+DHA levels was observed, but without any statistical significance.

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Table 1. Lipid sources, expected EPA+DHA and estimated variation in formulation cost of the diets.

	CTRL	SARDINE	ALGASALMON	ALGARAPE	ALGASOY	ALGABLEND
Ingredients (%)						
Sardine oil	5.0	8.5	-	-	-	7.9
Rapeseed oil	8.0	4.5	-	8.6	-	4.7
Soybean oil	-	-	-	-	8.6	-
Salmon oil	-	-	9.9	-	-	-
Algae oil	-	-	3.1	4.4	4.4	-
Blend of algae	-	-	-	-	-	2.0
EPA+DHA (%)	2.0	3.0	3.0	3.0	3.0	3.0
Increased cost (%)	-	6	16	17	17	41

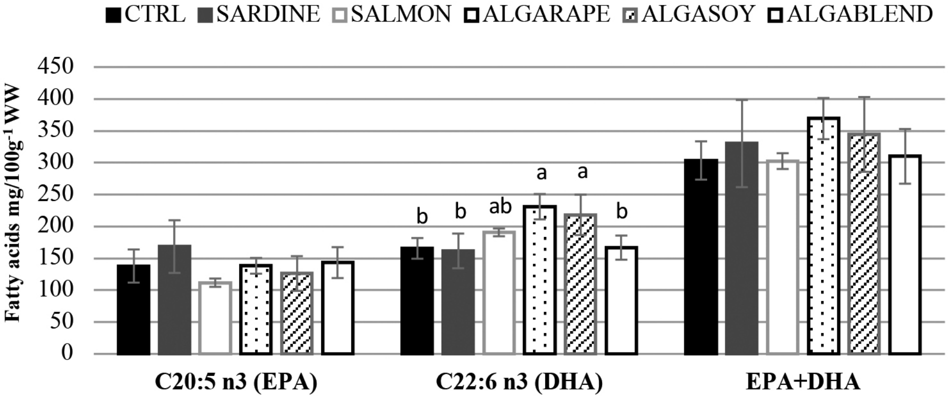

Figure 1: Muscle EPA, DHA and EPA+DHA content of sea bass fed the experimental diets for 54 days. EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid. Values are presented as mean ± standard deviation (n = 4).

Discussion and Conclusion

The present study shows that it is feasible to include alternative lipid sources to increase the sustainability of diets for sea bass, without affecting growth performance and nutrient utilisation.

Taking into account the final muscle content of EPA and DHA, it can be concluded that the combination of pricy n-3 LC-PUFA sources with more economic and/or eco-friendly lipid ingredients, such as salmon by-product oil and algae is a viable solution for replacing sardine oil in feeds for European sea bass. Overall, the present study showed the feasibility of achieving good levels of DHA and EPA in the final product.

Acknowledgements

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IMTA FOR THE FINAL EFFLUENT TREATMENT AT THE FIRST TUNA AQUACULTURE PRODUCTION FACILITY IN EUROPE

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Introduction

Next Tuna GmbH is planning, the first of its kind, production facility for Atlantic Bluefin Tuna (*Thunnus thynnus*) at the shore of the Mediterranean Sea. (see as well https://www.nexttuna.com/)

An essential part of Next Tuna's mission is to follow a sustainable production approach. A part of this approach is the application of an integrated multi trophic aquaculture (IMTA) system to treat/polish the run-off water from the state-of-the-art recirculation aquaculture facility.

Since the use of multi trophic nutrient extraction is mainly known as a marine add-on application to net-pen aquaculture, Next Tuna wants to apply this technology in a more concentrated, intensive application under fully controlled conditions.

Approach

Based on a desktop study, the work is focusing exclusively on the IMTA as application for final effluent polishing and nutrient extraction prior to effluent discharge into the sea. The expected incoming loads (volumes and nutrient concentrations) are given and based on long term experience with RAS. The available space for the IMTA application within the Next Tuna production facility is already earmarked and needs to be filled with the most suitable concept. The recirculation aquaculture part of the production facility is only seen as the source of nutrients for the IMTA. The added value of the IMTA production and its costs are discussed but are not the main scope of this work.
The scope of work is to identify:

1. The right combination of primary production species (algae or higher plants) and the suitable filter feeding shellfish for the multi trophic application based on available literature and ongoing international projects on IMTA.
2. Based on the findings form point 1, develop a system layout and calculate expected effluent nutrient concentrations based on known incoming nutrient loads.

Results

Within the framework of the project we:

1. Identify the most suitable combination of plants and shellfish for the planned application
2. Dimension a most suitable system for the most effective nutrient extraction under the given circumstances.
3. Calculate expected values of nutrient concentration after the IMTA application.

Discussion

The developed intensive IMTA application is benchmarked against experiences and expectations known for net-pen applications of the IMTA approach.
The intensive form of IMTA application is discussed with potential risks and management recommendations.

SAFE SEAWEED BY DESIGN: SUPPORTING THE SEAWEED SECTOR TO ENSURE SEAWEEDs ARE SAFE FOR HUMAN CONSUMPTION and THE ENVIRONMENT

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Introduction

The increased global demand for food and feed combined with a changing climate confronts societies with the challenge to explore new sources of food and feed. In recent years, there has been significant interest in the potential contribution of seaweeds to the future food systems.

The growth of the global seaweed sector, already a source of income to farmers in moderate and tropical regions and now expanding to other regions, is fuelled by the development of (new) applications of seaweeds in food, feed, cosmetics and pharmaceuticals (Buschmann et al. 2017). Seaweeds not only can help meet future food demand – locally and abroad – they can also play an important role in stimulating food production on land and at sea, and contribute to the provision of ecosystem services, including climate mitigation. On land, marine resources can assist with improving soil quality and terrestrial production system efficiency, for both crops and livestock (Abbott et al. 2020; Seghetta et al. 2017). At sea, marine resources can increase nursery habitats and provide valuable ecosystem services such as CO_2 and mineral fixation, nutrient recycling, and long term carbon storage (Froehlich et al. 2019; Duarte et al. 2017).

It is crucial to ensure the further development of the seaweed sector is safe, for human consumption, for the environmental and for workers, as concerns are raised about the impact of large scale cultivation on the marine ecosystem (van der Meer, 2020), food safety (Banach et al, 2020).

Objective

This study aimed to support the development of a safe seaweed sector by providing protocols for safe seaweed production. Based on literature review, interviews with experts and a pilot, the Safe Seaweed by Design project aims to deliver science-based safety protocols. The protocols identify key risks and support the selection of appropriate methods for risk assessment. The study concludes with recommendations for business and government to advance the role seaweeds.

Methodology

The study pulls together information from various data sources. The first phase involves a review of literature on risk associated with seaweed production. This review was validated with expert interviews and a survey. Subsequently, the study teams developed and tested draft protocols in a pilot, conducted in Norway.

Intended output

The results from the study will support development of seaweed application in future food systems. Protocols are designed to be taken up by the sector, including seaweed producers and processors.

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MODELLING SEAWEED CULTIVATION ON THE DUTCH CONTINENTAL SHELF

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Introduction

While Saccharina latissima seaweed is currently cultivated in the Netherlands at a small scale, there is a major drive towards increasing sustainable offshore cultivation. Future wind farm areas are seen as potential development locations. Figure 1 indicates the operational wind farms and designated areas for wind energy development at the time of the start of this research (development areas have meanwhile changed).

Harvesting seaweed leads to nutrient removal from the ecosystem. If this is done on a large scale, this can subsequently affect phytoplankton primary production, the basis of the marine food web. In this study, we investigate the spatial variability in ecosystem responses to seaweed cultivation in the Dutch North Sea in designated wind development areas. We focus on the interaction of seaweed and phytoplankton productivity with respect to nutrients and the effect of size, location and distribution of farms.

Approach and results

We integrated a module simulating seaweed nutrient uptake and growth dynamics into a fully coupled 3D hydrodynamic-water quality model of the North Sea. We ran different cultivation scenarios over a production year and compared them to a run without seaweed cultivation. Results showed that offshore locations are slightly more productive for seaweed cultivation than those closer to the coast, and that it is beneficial to split the cultivation area into relatively small plots. Growth of total seaweed biomass is mainly driven by temperature and light, since the fronds do not run out of reserves before harvest. Extreme upscaling of seaweed production to 25% of all Dutch designated offshore windfarm areas would have a strong impact on the ecology, with local decreases in spring phytoplankton production as high as 30%. The effect on nutrient concentrations and phytoplankton primary production is directly related to the size of the farms. Even at this stocking density, target yields were still achieved, indicating that production carrying capacity is not yet reached. However, at this level it is likely that there is an effect on ecological carrying capacity. What is acceptable or desirable in terms of nutrient removal and impact on phytoplankton primary production requires a societal debate, but models such as these can feed this discussion.

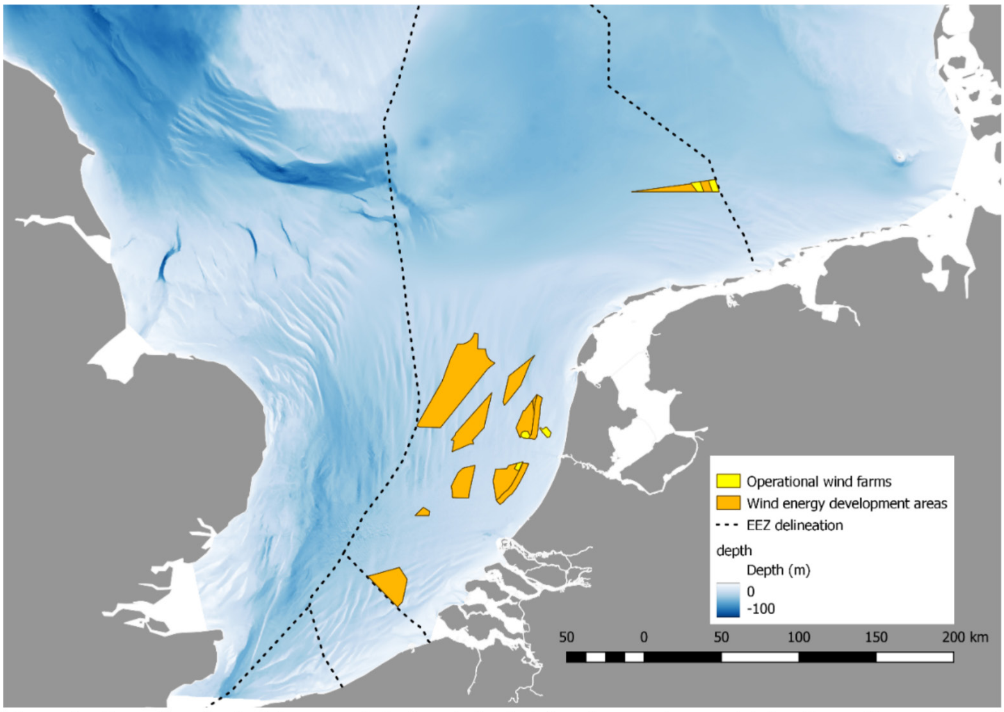

Figure 1: Operational wind farms and designated wind development areas (at the time of carrying out this research).

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The effects of large farms can extend far downstream. For example, seaweed cultivation over ~25 km2 at Borssele leads to a decrease in spring primary production >5% over more than 3,000 km2, all the way North from the Dutch Wadden Islands. Therefore, the location of seaweed farms with respect to each other is also an important factor to consider when upscaling seaweed production. Placing a new seaweed farm in the area of influence of another one can lead to higher local reductions in nutrient concentrations and phytoplankton primary production.

Outlook

The scenarios presented here are hypothetical. When sufficient field data is available, the seaweed module should be further calibrated and validated for Dutch waters to endorse the reliability of its predictions. This model could then be used to refine our estimates of the ecological carrying capacity of the Dutch North Sea for seaweed cultivation.

"THE USE OF SOY PRODUCTS AS A REPLACEMENT FOR FISHMEAL IN AQUA DIETS "

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On a world-wide scale it is estimated that fed aquaculture supplies 125 MMT or 70 % of the total aquatic production. It is expected that this production, as well as the proportion of total aquaculture production, will increase with approximately 2 % per year until 2030 (OECD/FAO, 2021). The associated annual production of commercial aqua feed is estimated at more than 51 MMT and fish meal production is estimated at 5.4 MMT (OECD/FAO, 2021). The supply of fishmeal - an ingredient considered essential in many aqua diets - is limited, leading to high prices of the fish meal and consequently the feed produced with it. Alternative sources of protein (amino acids) have been considered and tried in a large range of diets. These include animal co-products and plant proteins; principal among the latter is soy since it is the main plant protein being produced and its nutritive value in terms of amino acid profile is most advantageous for most aquatic species.

On a global scale, production of soybeans has increased steadily for more than 100 years reaching close to 370 MMT/yr. (OECD-FAO, 2021). Soybeans are crushed and converted in many ways leading to many different types of soy products that can play an important role in replacing part - or all - of the fish meal in aqua diets.

The main soy co-product is soybean meal (SBM) with an estimated production of more than 265 MMT globally. SBM has been – and is - extensively used in fish feed. In omnivorous fish, accounting for more than 70 % of the global aquaculture volume (Nailor et al., 2021), plant proteins - most notably SBM – have largely replaced fish or animal proteins. In certain species such as carp, catfish and tilapia, SBM may represent as much as 60 % of the total formula (Gatlin, 2013) thus covering almost all of the protein requirements. Soy products have also successfully been included in diets of carnivorous fish where they have been able to replace variable proportions of fish and animal proteins. This includes specific diets for trout as well as various marine fish diets (Powell et al., 2016; Kotzamanis et al., 2020; Velez-Calabria et al., 2021.

Such relatively high levels of SBM are possible, primarily due to SBM's relatively high levels of crude protein and amino acids but also because of its high protein (amino acid) digestibility and its adequate palatability. Among all plant proteins, soy products are recognized for having one of the most balanced amino acid profiles, although their content in sulfur-containing amino acids methionine and cystine may be limiting compared to the established, quantitative amino acid requirements of most fish species (NRC, 1993). Variable amino acid supplementation of soy-containing diets is needed and high-level replacement of FM by SBM (up to 90 %) has been successfully demonstrated in trout diets supplemented with valine, lysine, methionine and taurine without apparent decrease in growth or intestinal health status. (Velez-Calabria et al., 2021). Similar responses with additional improvements in carcass quality have been obtained with supplemental taurine in specific marine species (Kotzamanis et al., 2020). As a matter of fact, in many species the replacement of fish or animal proteins by soy proteins is hampered by an inadequate understanding of the digestible amino acid requirements for these species and age groups within species.

In the case of properly crushed SBM, amino acid digestibility is generally high although considerably differences among species have been shown. In fish and shrimp the digestibility of the soy protein fraction has been found to be high and generally close to 90 %, (e.g. Gatlin, 2013; Brezas and Hardy 2020; Kumar et al., 2020; Galkanda-Arachchige et al., 2020). Much of the variation in digestibility of SBM is related to quality and especially the degree of heat treatment.

Following a correction for the specific amino acids, higher levels of inclusion of soy products in fish and shrimp diets are limited by the presence and possible effects of the residual anti-nutritional factors (ANF). This is especially true for fry and juvenile fish which are exceptionally sensitive to ANFs. These components – present to variable degrees in soy product – have been associated with intestinal inflammations (enteritis) in commercial fish species leading to reduced absorptive capacity, increased mucus secretion, hyperpermeability, and leucocyte infiltration. Directly or indirectly, they have also been related to pro-inflammatory cytokine genes expression and changes in the composition and population of gut microbiota through the supply of nutrients that preferentially support or depress the presence of specific gut bacteria (Kumar et al., 2020).

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Partial removal of the heat labile ANF is achieved through toasting or expansion/extrusion. Heat resistant ANFs require alternative methods of treatment such as extraction (as in the case of SPC or ISP). An alternative, efficient and economical method for ANF reduction has proven to be the fermentation of SBM; alone or in combination with enzymatic extraction (Mukherjee et al., 2016; Zhang et al., 2021). These methods, most notably the solid-state fermentation of SBM (FSBM), are rapidly gaining interest allowing the inclusion – or increase in inclusion – of soy in aqua diets. This is especially important for carnivorous fish and shrimp where inclusion of FSBM allowed successful replacement of fish meal and improved performance. Production of FSBM may vary considerably by type of inoculum used and conditions of fermentation. However, these differences in production have not shown consistent, demonstratable differences in performance.

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"THE USE OF SOY PRODUCTS AS A REPLACEMENT OF FISHMEAL IN AQUA DIETS "

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THE TRANSCRIPTOMIC RESPONSE OF ATLANTIC SALMON *Salmo salar* TO MICROALGAE DIETS AND ENVIRONMENTAL STRESS

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Introduction

Atlantic salmon (*Salmo salar)* is an anadromous salmonid of high economical value globally. Since it is a nutritious food product, Atlantic salmon contributes significantly to economic and employment security in many countries worldwide. With aquaculture being one of the most rapidly expanding food production sectors, the global salmon production in 2019 reached approximately ~2.6 million tons. For salmons, entry into seawater is a crucial part of their life cycle and a critical step in production. A high pathogen pressure in the marine environment combined with a suppressed immune system of the fish results in a higher susceptibility to infections and diseases. Fish welfare is recognized to be essential for high product quality and a good health status. Stressed fish commonly have poor health and performance. Monitoring fish health status by measuring gene expression of biomarkers could be a useful tool to take measures for stress reduction.

Microalgae are considered promising functional feed ingredients, as they have beneficial effects on immune status. However, knowledge on the immune and stress effects of microalgae diets in Atlantic salmon remains limited. Previous research revealed the serious impact feed additives can have on gut health and systemic physiological parameters in salmonid fish. This study aims to investigate the influence of microalgae diets as probiotic feed and the transfer into marine farming systems on the expression of genes functioning as biomarkers for fish health status.

Material & methods

Atlantic salmon were randomly assigned to 18 experimental tanks (28 smolts per tank) in a recirculating aquaculture system (RAS) of Aquaculture Research Station Büsum, Germany. The tanks were filled with brackish water (~ 12 psu). After 8 weeks, the fish were transferred from brackish water to seawater (~ 32 psu). The fish were fed manually to apparent satiation twice daily. The diets were supplemented with different microalgae (8%). The microalgae used were *Spirulina, Chlorella broken, Schizochytrium, Chlorella intact* and *Tetraselmis*. In order to analyze the fish health status, RNA was isolated from five different organs (head kidney, spleen, gills, intestine, liver). Subsequently, the Biomark HD/Fluidigm test system was used for multigene expression analysis.

Results

In our study, we observed inter-individual differences in gene expression between fish fed with the same microalgae diets. In addition, following expectations, the different immune-related organs showed a different immune response. Overall, we recorded no major down- or upregulation of immune-related genes.

Discussion and conclusion

The present study demonstrates that microalgae do not have adverse influences on the immune status of Atlantic salmon and could serve as feed additive in the feeds of Atlantic salmon.

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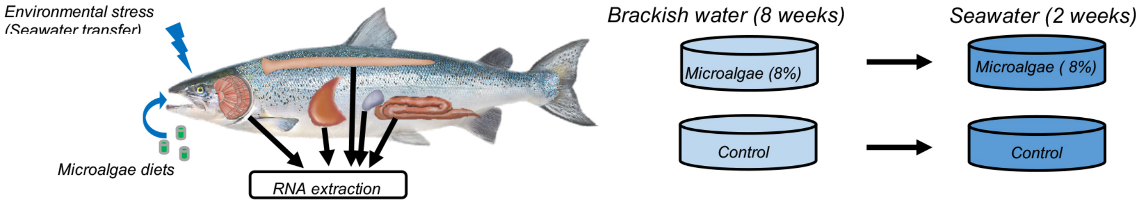

Fig.1. **Schematic representation of the experiment.** The Atlantic salmon was kept for 8 weeks in brackish water and subsequently transferred for 2 weeks in seawater. The fish were fed with microalgae supplemented (8%) diets throughout the whole experiment. In parallel to 5 different microalgae used for the diets, we had one control group. After the trial, the gills, liver, head kidney, spleen and intestine were sampled in order to isolate the RNA and perform a multigene expression analysis.

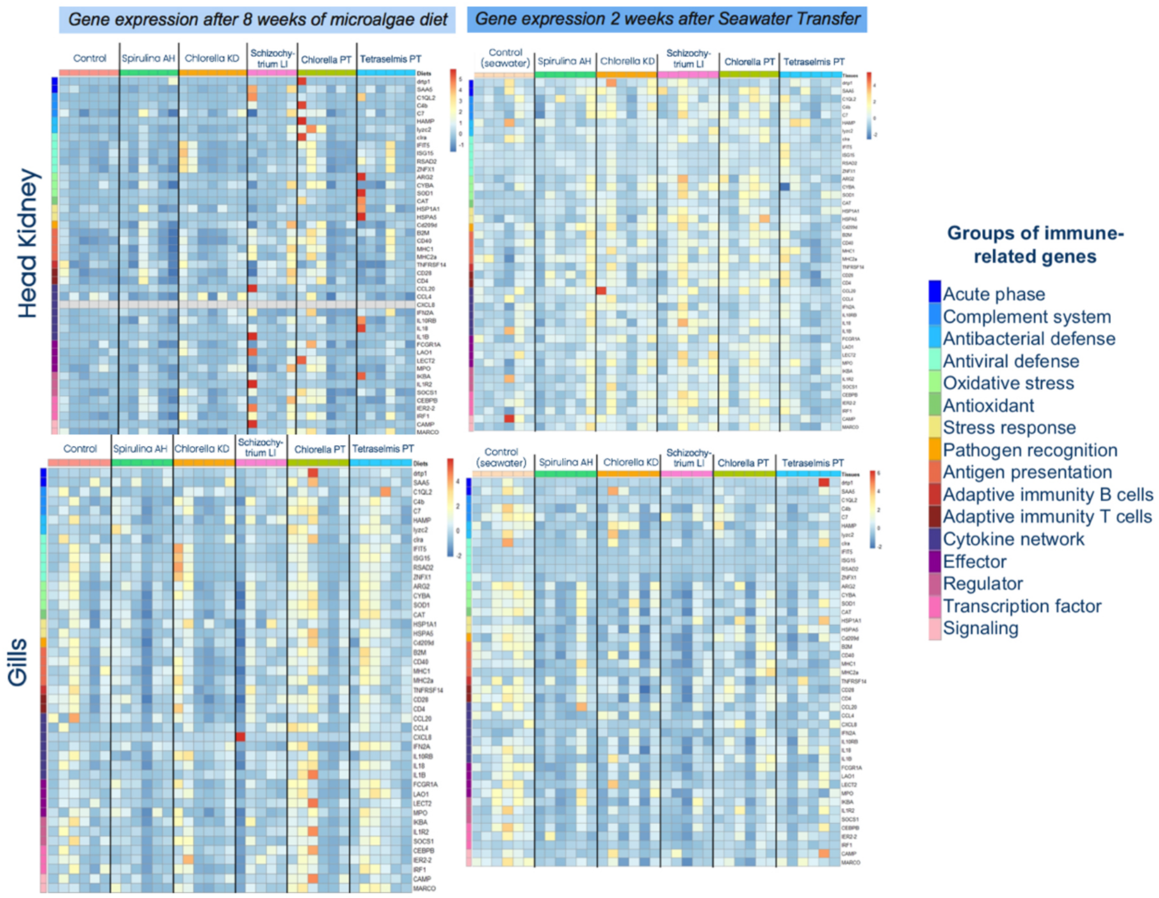

Fig.2. **Heatmaps of gene expression in the Head Kidney and Gills.** On the left side, the results after 8 weeks and on the right side 2 weeks after SWT.

SEATACROPS: HEALTHY AND SUSTAINABLE FOOD FROM ON LAND SEAWEED CULTIVATION IN BELGIUM (in prep.)

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Introduction

There is a rising demand for alternative and sustainable food sources such as seaweed. In order to secure tomorrow's food the European Union is concerned how we can obtain more biomass from the ocean without depriving future generations of their benefits. Seaweed (or macroalgae) are low-trophic marine organisms and meet the criteria when it comes to low environmental impact when cultivated and for their variety of health benefits for humans and animals. Due to the high level of fibres, proteins, minerals (Ca, K, Fe, …), vitamins (B1, B12, …) and antioxidants seaweed would be a good addition to our current diet. According to the Food and Agriculture Organization (FAO), protein required by Europe in 2054 will be around 56 million metric tonnes. Seaweed-derived protein may offer a viable solution. For seaweed to grow, it does not compete with conventional agricultural land and it does not require fresh water and pesticides. In comparison with at-sea cultivation, where seasonality has a huge impact on the quality and quantity of the production, on land cultivation can deliver year-round production of high-value products that are suitable for food applications.

Materials and Method

The red algae *Palmaria palmata* and *Porphyra sp*. were collected from the wild and kept in closed cultivation systems. A series of experiments on lab scale (1 L bottles) and small scale (40 L buckets) were conducted to obtain optimal cultivation conditions (light intensity, light quality, day length, water movement, stocking density and nutrient concentration) in order to maximize the specific growth rate (SGR) of the seaweeds.

Based on the findings from the lab and small-scale experiments a recirculating aquaculture system (RAS) was developed for the cultivation of *P. palmata*. The system consisted of three interconnected tanks with a volume of 1 m^3 each, a first step towards commercial production. The seawater was recycled continuously with a turnover rate of 2400 % day^{-1}. Water exchange was practically zero, except for a small amount of water that was added weekly to compensate for evaporation. UV was used to supress the outgrowth of micro-organisms. On a weekly basis the seaweeds were checked for quality, weighted and growth media was added to the system. In between two measuring days, the system operated autonomously and was followed up remotely by continuous measurements of salinity, temperature, dissolved oxygen and pH using submerged probes. Macro-nutrients in the water, such as nitrate, phosphate and ammonium are essential in the field of aquaculture, but unfortunately they remain hard to measure analytically or require expensive apparatus. In order to be able to follow up the nutrient uptake kinetics of the seaweeds and better understand their nutrient requirements an inexpensive autonomous in situ wet chemical phosphate detector was developed and tested.

Results

On a lab scale maximum growth rates of 8 % day^{-1} and 10 % day^{-1} for *P. palmata* and *Porphyra sp*. were reached, respectively. Under small-scale conditions different strains of *P. palmata* showed similar growth rates. However, the natural cycle of the seaweed (alteration between growth and forming new proliferations) introduced a lot of variation in the data ranging from 2 to 8 % day^{-1}. In the RAS *P. palmata* gave average yields of 600 ± 17.1 g $week^{-1}$ m^{-3} or 3 ± 0.1 % day^{-1}.

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Discussion and conclusion

The high growth rates of 8 % day^{-1} that were obtained in the lab and small-scale experiments with *P. palmata* could not be repeated in the RAS. By further optimizing tank dimensions, water circulation, nutrient requirements and light conditions we hope to reach higher yields in the future. Since we now better understand the natural cycle of the seaweed we can exploit this knowledge and develop a cultivation scheme that is adapted to this. Furthermore, we are working on developing new hatchery techniques in order to close the life cycle of both *Palmaria palmata* and *Porphyra sp.* to become self-sustaining and no longer dependent and wild populations. And finally, the idea is to further explore the possibilities for automatization of the RAS system, so labour and energy costs can be reduced significantly.

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CAN WE COMBINE GENETICS AND A TWO-STEP TEMPERATURE CONTROL TO MOVE TOWARDS MONOSEX EUROPEAN SEA BASS *Dicentrarchus labrax*?

Vandeputte M.[1], Clota F.[1], Vergnet A.[1], Blanc M.O.[1], Lallement S.[1], Ruelle F.[1], Sánchez-Baizán N., Besson M., Piferrer F., Cousin X., Goikoetxea A.[1], Leitwein M.[1], Allal F.[1] and Geffroy B.[1]

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Introduction

The sea bass, *Dicentrarchus labrax,* is an important species for European mariculture, mainly in Mediterranean countries. In this species, females grow faster and reach a higher weight than males. However, most farmed fish batches show a highly male-skewed sex-ratio. The sea bass has a complex sex determination system, combining both genetic (polygenic) and environmental (temperature) influences. Low initial temperatures (<17°C) favor female sex determination. In contrast, temperature in the late post-larval phase has the opposite effect, with cold temperature favoring male differentiation and high temperature (up to 23°C) favoring female differentiation (Clota et al., 2021). However, data on the combined effects of genotype and temperature are lacking. We need to know whether there is genotype-by-environment (GxE) interaction or not, to deploy a strategy towards producing monosex female individuals for production while keeping a balanced sex-ratio for hatchery and selective breeding.

Results

Starting at 1 day and using incremental rearing periods at 16°C (31 to 244 days), followed by an increase to 21°C, we first showed that, while 31 days at 16°C led to 26% of females, increased cold exposure promoted the proportion of females, up to 46% after 74 days at 16°C. Conversely, exposure to 16°C for a period longer than 74 days progressively reduced the proportion of females, reaching a minimum of ≈ 10% after 230 days at 16°C. In a second experiment, we demonstrated that after an initial rearing of fish for 90 days at 16°C, a secondary exposure to four different temperatures (19, 21, 23, 25°C) resulted in different sex-ratios, with more females at higher temperatures (from 30% at 19°C to 49% at 25°C). Then, we examined the genotype-by-environment interaction in both periods, by genotyping fish with a 57K SNP chip, comparing 1) two groups reared at 16 or 21°C during the first 60 days and 2) four groups of fish reared at 19, 21, 23 or 25°C after 90 days at 16°C. The genetic correlation of sex tendencies between the two early temperature groups was very high (0.91 ± 0.09). In the late temperature treatments, the genetic correlations of sex tendencies between the first three temperature groups (19, 21 and 23°C) were close to unity (0.98± 0.16 to 1.00± 0.16), showing little, if any, GxE interaction. Conversely, the genetic correlations of sex tendency at 19, 21 and 23°C with sex tendency at 25°C were lower (0.82 ± 0.21 to 0.91 ± 0.21). Thus, genomic predictions of sex tendency can be reliably performed using data from any temperature treatment, provided the late temperature (after 90 dph) is not higher than 23°C.

The way forward

Based on these findings, we propose a method combining temperature treatments and genomic evaluation of sex tendency to 1) identify individuals with a strong “female” genetic background, including “neomales” sex-reversed by temperature treatments, and 2) use them with adequate temperature treatments to obtain near-monosex batches.

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PHLOROGLUCINOL TREATMENT INDUCES TRANSGENERATIONAL EPIGENETIC INHERITED RESISTANCE AGAINST *VIBRIO* PARAHAEMOLYTICUS IN A BRINE SHRIMP MODEL

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Introduction

Emerging, infectious diseases in shrimp like acute hepatopancreatic necrosis disease (AHPND), caused by *Vibrio parahaemolyticus* are worldwide related to huge economic losses in industrial shrimp production. There is an urgent need to develop innovative AHPND preventive methods that also support sustainable shrimp aquaculture.

Exposure to non-lethal heat shock (NLHS) of Pacific white shrimp (*Litopenaeus vannamei*) and brine shrimp (*Artemia franciscana*) induced the expression of endogenous heat shock proteins and activated the innate immune system, resulting in enhanced disease resistance against *Vibrio* infections. Thus, exposure to NLHS might be a strategy for protecting animals and their progenies against AHPND and possibly other infections. However, its practical application in industrial shrimp farming is cumbersome. Also, temperature shifts are often detrimental and can negatively affect the physiological and immunological balance. Therefore, the administration of Hsp-inducing compounds as an alternative to NLHS exposure has been proposed.

Kumar et al., (2018) showed that the plant derived polyphenolic compound phloroglucinol perhaps might be used as a biocontrol agent as it increased the resistance against *V. parahaemolyticus* AHPND infection in axenic brine shrimp and the giant freshwater prawn (*Macrobrachium rosenbergii*) by inducing endogenous Hsp70.

In the current study, using brine shrimp as an animal model, we tested whether phloroglucinol exposure of the parental generation at early life stages could induce heat shock proteins and elicit an AHPND- resistant phenotype in the offspring.

Materials and methods

Parental brine shrimp were treated with phloroglucinol, starting from day after hatching 1 (DAH1) until DAH16, to ensure that the uterus, which normally develops by 3 weeks and carries the cysts/embryos, was not directly exposed to the compound. Controls were untreated (Fig 1).

Adult parental females (CF0 and TF0) produced next-generation CF1 and TF1 larvae and, under suboptimal conditions, CF1 and TF1 cysts. Adult CF1 and TF1 females produced CF2 and TF2 larvae and, under suboptimal conditions, CF2 and TF2 cysts. Subsequently, adult CF2 and TF2 females produced CF3 and TF3 larvae and, under suboptimal conditions, CF3 and TF3 cysts. The F1, F2, and F3 larvae and cysts were never exposed to phloroglucinol.

Next, all cysts were hatched simultaneously for a common garden experiment. The age- and size-synchronized axenic 1-day old brine shrimp larvae were used for: a *V. parahaemolyticus* challenge assay, immune gene expression analyses (RT-qPCR), DNA methylation assays and histone modification assays.

Results

Phloroglucinol treatment of parental brine shrimp significantly increased the resistance of their progeny for 3 subsequent unexposed generations (F1, F2 and F3) against bacterial infection with a highly virulent *Vibrio parahaemolyticus* AHPND strain.

Challenged (AHPND) offspring (TF1 and TF2) originating from treated parents showed a significant upregulation of immune-related genes compared to challenged offspring from non-treated parents. We found no link between global DNA methylation and the increased resistance in offspring against AHPND infection. However, H3 hyper-acetylation and hyper-methylation probably played a role in transgenerational (F1, F2) inheritance of the phloroglucinol-induced, AHPND disease resistant phenotype. Results showed that epigenetic reprogramming is likely to play a role in the underlying mechanism.

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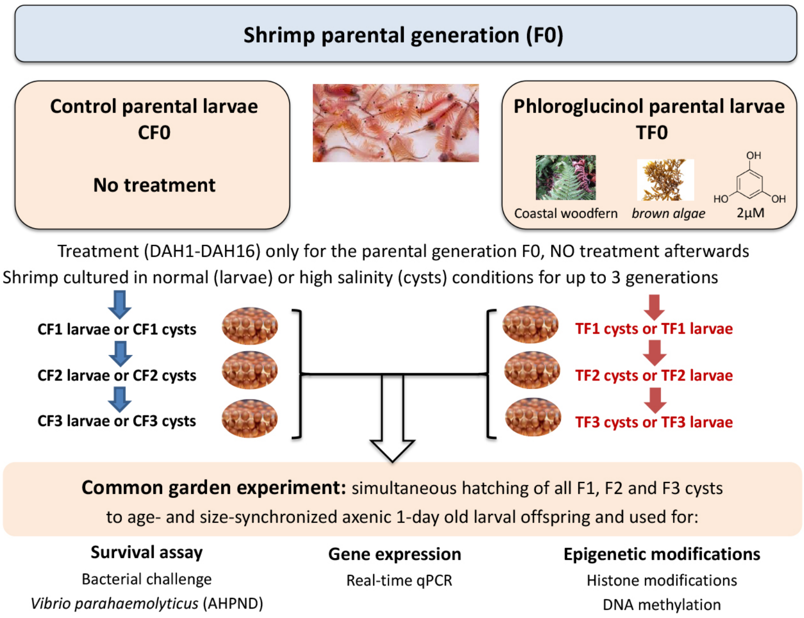

Figure 1. Schematic overview of the experimental setup.

Conclusions

Here, using axenic brine shrimp (*Artemia franciscana*) as a model system, we showed that phloroglucinol treatment of the parental animals at early life stages resulted in transgenerational inherited increased resistance in their progeny against *V. parahaemolyticus* AHPND infection. In the future, parental priming might be used for brood stock management to produce more robust, disease resistant progenies in crustaceans. The principle of trained immunity might be used in shrimp aquaculture as a novel strategy for creating broad-spectrum protective innate immune response against economically important pathogens

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USE OF EXOGENOUS ENZYMES TO IMPROVE THE NUTRITIONAL VALUE OF FORAGE LEGUMES AS FEED INGREDIENTS IN EUROPEAN SEA BASS NUTRITION-*IN VITRO* EVALUATION

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Introduction

The expansion of aquaculture production has been associated with the need of rapid growth in aquafeed production. Forage legumes are promising candidates and highly valued feed components for both ruminants and monogastric livestock due to their relatively high protein, balanced amino acid profile and energy values compared to cereal grains. However, their use in feed is still limited by the presence of several antinutritional factors (ANFs) that decrease enzyme activity and the absorption of minerals and other nutrients (Francis et al., 2001). Exogenous enzymes have the ability to improve the digestibility of many ingredients, by breaking down the complex cell wall structure that encapsulates nutrients presents in plant based feed. Aim of the present study was the *in vitro* evaluation of forage legumes treated with different processes with exogenous enzymes and conditions.

Materials and methods

Lupinus albus, *Cicer arietinum* and *Favino faba* whole seeds were grinded to produce legume meals, and evaluated for their nutritional value. Samples were treated under different condition processes (moisture, temperature, time and pH) and enzyme combinations. Briefly, 4 commercial enzyme products were assessed i.e. xylanase, phytase and two multienzymes complexes. Two different moisture levels (8 and 45%) and thermal treatments (25 and 50°C) were evaluated. Process duration was either 25 or 240 min and in selected processes pH was reduced with the addition of citric acid. All legume products were evaluated for their protein content after treatment, trypsin inhibitors (TUI) (Liu et al., 2021), phytic acid and free phosphorus (P) (spectrophotometrically).

Results and discussion

ANFs combine with nutrients and act as inhibitors by reducing nutrients bioavailability. Specifically, phytic acid hinders the activity of enzymes, which are necessary for protein degradation in the intestine and stomach (Kies et al., 2006). Phytic acid normally constitutes 0.2%–2% of the dry weight in cereals and legumes with 60%–90% of the total phosphorus present bound to phytate (Feizollahi et al., 2021). The use of exogenous enzymes increase digestibility of nutrients and bioavailability. Additionally, cooking of pulses is a commonly used process that highly improved the nutritional value of foods by reducing their ANFs (Patterson et al. 2017). The results showed positive effect of enzymes, thermal treatment, high moisture level and acidic pH in nutritional and ANFs examined (Table 1). In *Lupinus albus* meal multi-enzyme product along with high moisture level (45%) and thermal treatment (50°C) resulted in lower trypsin units inhibited (0.40 TUI/mg sample) and phytic acid content (0.23%), while free P increased from 0.08 to 0.34%. *Cicer arietinum* showed lower TUI/mg sample in same treatment conditions and enzyme process as *Lupinus albus*. However, free P was higher and phytic acid lower when phytase-xylanase was added. Treatment with a combination of phytase-xylanase had positive effect in *Favino faba* meal reducing TUI from 4.38 to 0.20 /mg sample. Moreover, free P content increased with the same enzymes and treatment conditions but only in acidic pH (5.5).

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Table 1: Nutritional and ANFs in legumes with different processes and enzymes (dry sample).

	Treatment										
Legume	**Moisture (%)**	**T (°C)**	**pH**	**t (min)**	**Multi-enzyme**	**Xylanase - protease-amylase**	**Phytase-xylanase**	**TUI/mg sample**	**Free P (%)**	**Phytic acid (%)**	**Protein (%)**
Lupinus albus	8	25	5.5	30				1.47	0.08	1.31	40.8
					+			0.97	0.11	1.18	41.0
						+		0.55	0.10	1.24	39.9
							+	0.45	0.19	0.96	40.4
	45	50	5.5	240	+			0.40	0.34	0.23	41.7
						+		0.90	0.20	0.74	41.6
							+	0.30	0.28	0.54	40.9
Cicer arietinum	8	25	6.4	30				14.39	0.08	0.96	24.1
					+			13.08	0.10	0.91	23.8
						+		13.04	0.09	0.94	23.7
							+	13.84	0.16	0.66	23.9
	45	50	6.4	240	+			0.29	0.16	0.57	24.2
						+		0.24	0.31	0.08	24.1
							+	0.18	0.29	0.09	24.1
			5.5		+			0.16	0.29	0.15	24.0
						+		0.18	0.17	0.52	24.0
							+	0.29	0.32	0.01	23.8
Favino Faba	8	25	6.4	30				4.38	0.09	1.25	28.6
					+			2.49	0.10	1.28	28.4
						+		2.49	0.09	1.26	28.7
							+	2.36	0.20	0.87	28.7
	45	50	6.4	240	+			0.21	0.36	0.15	28.8
						+		0.24	0.15	0.85	28.8
							+	0.20	0.24	0.53	28.9
			5.5		+			0.29	0.36	0.17	28.6
						+		0.37	0.20	0.68	28.3
							+	0.30	0.40	0.22	28.4

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POTENTIAL ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF HYDROLYSATES FROM FISH PROCESSING BY-PRODUCTS

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Introduction

The fast growth of aquaculture together with the limited availability of dietary fishmeal (FM) has forced the feed industry to search for alternative and more sustainable dietary protein sources. To face the challenges of replacing increasing levels of FM by plant protein sources, low levels of plant- and animal-protein hydrolysates can be incorporated into fish diets, providing bioactive peptides with benefits beyond their nutritional value, including antioxidant, immunomodulatory and antimicrobial activities (1). Currently, fish processing industries discard large quantities of fish skin and bones. These by-products can be valorised and originate new added-value products with functional properties, contributing to a circular economy (2). In order to select the best candidates to be included in aquafeeds, the aim of the present study is to have an in-depth comprehension of the bioactive potential of distinct hydrolysates obtained from fish processing by-products.

Materials and methods

Discarded skin of blue shark (*Prionace glauca*) was processed in order to obtain 3 different fractions: 1) total skin (TS); 2) a collagenic fraction (C); and 3) a non-collagenic fraction (NC). Each fraction was then hydrolysed using two different enzymes (alcalase and bromelain), resulting in 6 different hydrolysates: 1) total skin hydrolysed by alcalase (TSHA), 2) total skin hydrolysed by bromelain (TSHB), 3) collagenic fractions hydrolysed by alcalase (CHA), 4) collagenic fractions hydrolysed by bromelain (CHB), 5) non-collagenic fraction hydrolysed by alcalase (NCHA), 6) non-collagenic fraction hydrolysed by bromelain (NCHB). These nine experimental fractions (hydrolysed and non-hydrolysed) were analysed *in vitro* to unreveal their antioxidant capacity through 2′-azinobis-3-ethylbenzthiazoline-6-sulphonic acid and oxygen radical absorbance capacity ($ABTS^{+\bullet}$ and ORAC, respectively). The antimicrobial activity of hydrolysates was also tested *in vitro* by assessing the bactericidal and bacteriostatic potential against several pathogenic bacteria with high impact on fish health, well-being and aquaculture production (*Vibrio anguillarum, Vibrio harveyi, Vibrio parahaemolyticus, Aeromonas hydrophila, Yersinia ruckeri, Edwardsiella tarda, Tenacibaculum maritimum, Photobacterium damselae*). All the results obtained from each hydrolysed fraction were compared with the non-hydrolysed one.

Results and Discussion

Overall, all hydrolysates and non-hydrolysates fractions displayed good bactericidal activity against the bacteria tested, excepting for *E. tarda,* which was killed just by CHB. On the other hand, only NC was not effective against *Y. ruckeri*. Regarding *V. parahaemolyticus,* the three fractions hydrolysed by bromelain (TSHB, CHB and NCHB) displayed the highest capacity to kill this bacterium (98, 90, 78%, respectively). For the bacteriostatic activity, the tested hydrolysates presented the highest bacteriostatic activity against *Vibrio* genera. Concerning the $ABTS^{+\bullet}$ assay, all fractions hydrolysed by alcalase displayed higher antioxidant capacity when compared with non-hydrolysed fractions and hydrolysates by bromelain. Similarly, hydrolysates by alcalase displayed the best results for ORAC assay.

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Conclusion

All fractions displayed between 10 and 98% of bactericidal activity against the species tested but were unable to kill *E. tarda* (expecting CHB*)*. Overall, enzymatic hydrolysates obtained by alcalase presented high bacteriostatic activity, while bromelain hydrolysates demonstrated the best bactericidal potential, with the most promising results being observed against *Vibrio* genera. Additionally, hydrolysates demonstrated high antioxidant capacity. Overall, the *in vitro* studies showcased those hydrolysates obtained from total skin, collagen and non-collagen fractions using the enzyme alcalase, are promising ingredients to include in aquafeeds. Moreover, the use of such functional feeds could promote both fish growth and health while being sustainable at social, economic, and environmental levels and contributing to the one health concept. But further studies *in vivo* are still warranted to ascertain the full potential of these products in aquafeeds.

Acknowledgements

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ECOLOGICAL INTENSIFICATION AND CIRCULARITY IN POND AQUACULTURE

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Pond food web and natural foods

Important advantages of pond systems include that they are self-cleaning, produce a significant amount of natural foods and recycle nutrients. These advantages are dependent on a well-functioning food web in the pond. To mineralize and recycles wastes through the pond's food web, sufficient energy (e.g. carbon (C)) and nutrients (e.g. phosphorous (P), nitrogen (N)), the latter in the right proportions, need to be available. When this is the case, waste accumulation will be minimal, and the majority of the fed nutrients will be retained in herbivorous/omnivorous species (e.g. tilapia, major carps, small indigenous fish species) grazing on natural foods. Feeding pelleted feed with a low (31% protein/kg DM) and high (24% protein/kg DM) C:N ratio in ponds, Nile tilapia fed the high C:N ratio feed consumed more natural foods, compensating for the lower nutrient content in the feed. Fish fed the high C:N ratio diet retained 87% of the fed nitrogen, compared to 59% in fish fed the low C:N ratio diet (Kabir et al., 2019, Figure 1).

Ecosystem approach to aquaculture and ecological intensification

According to the FAO ecosystem approach to aquaculture (Soto et al., 2008), ecological aquaculture is imbedded in the surrounding ecosystem which (cit.) 'contributes to sustainable development, equity, and resilience of interlinked social and ecological systems'. In an ideal society, ecological aquaculture occurs when the physical, production, social and ecological types of carrying capacity intertwine harmoniously (Weitzman and Filgueira, 2020) (Figure 2). In turn, numerous, more or less similar, definitions of 'ecological intensification' were proposed from a human utility perspective, focusing on (cit.) 'the use of ecological processes and functions to increase productivity, [that] strengthen ecosystem services and decrease disservices' (Aubin et al., 2019). Although not the intention, by defining ecological intensification in this way, there is a risk to downgrade the intrinsic value of the production ecosystem.

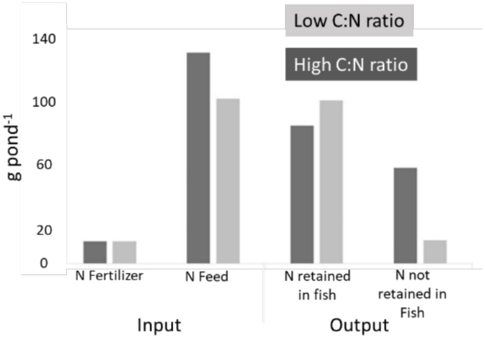

Fig 1: Effect of dietary C:N ratio on N input, N-output and N-balance in pond (modified from (Kabir et al., 2019)

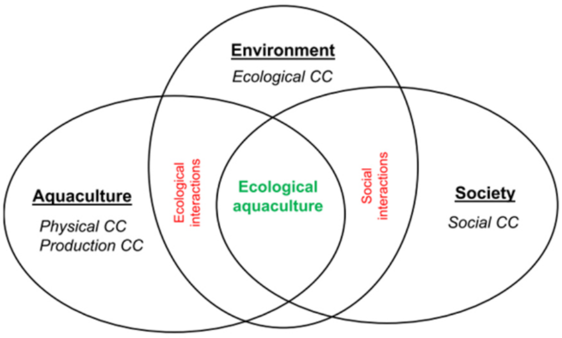

Fig. 2: How 4 types of **carrying capacity (CC)** jointly define ecological aquaculture. (Weitzman and Filgueira, 2020).

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Pond culture: a tool to recycle nutrients and optimize nutrient use efficiencies

To make aquaculture fully sustainable, more attention must be put of Ecological CC. A healthy pond ecosystem traps as much as possible bio-available macro and micro-nutrients and recycles them through the food web. During our experiments ponds retain more than 90% of the P input during the full culture period at harvest. The latter sometimes also happens for N. However, the amounts N and P retained in fish biomass turn out to be highly variable, fluctuating between 15 to 85%. Much more research is needed. At present, we lack sufficient insight to predict accurately optimal nutrient use efficiencies, including for nitrogen and phosphorous. Research should focus on recycling waste nutrients through the food web through culture organisms. Important research questions are how the types of feed influence on-farm nutrient use efficiencies and food web health, and how to maximize nutrient recycling through biomass gain of culture species in pond farming systems. The latter is our best bet to maintain the pond ecosystem productive and healthy and to contribute to food systems that produce withing planetary boundaries.

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DEVELOPMENT OF A GERM STEM CELL GRAFTING PROCEDURE IN EUROPEAN SEA BASS AS AN INNOVATIVE PRACTICAL APPROACH FOR BROOSTOCK MANAGEMENT

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Introduction

Enabling surrogate offspring production in fish is an emerging question and an interesting perspective for different objectives. Interspecific or intraspecific germ stem cell grafting may mitigate reproductive barriers, reduce generation time, help manage or recover broodstock, and enhance aquaculture productivity.

In this study, we explored the feasibility of germ stem cells (GSC) grafting in the European sea bass (*Dicentrarchus labrax*), a key European aquaculture species.

Material and Methods

We prepared two types of fish:

First, we produced 9 batches of sterile recipient sea bass larvae by triploidisation using the pressure shock method (Peruzzi et al. 2000). The ploidy level of the recipient fish was controlled by flow cytometry.

Second, 112 adult sea bass donors held in a recirculated system at constant temperature and photoperiod to avoid sexual maturation were sacrificed to recover the testis or ovary used to extract the GSC used in this study.

Preparation of the GSC was carried out by enzymatic and mechanical dissociation of gonads. Each tissue fragment was weighed and put in a digestive medium based on the cell culture medium L15 (Leibovitz) complemented by enzymes. Gonadal fragments were incubated in the digestive medium and placed at 20°C on an orbital agitator for 5h30. At two time points the pieces of gonads were mechanically dissociated by back and forth pipetting with a 10 ml pipette. Then, cells were centrifuged for 15 min at 500G and placed in a culture medium to stop digestion, and kept on an orbital agitator overnight. Just before the transplantation of the cells, tissues were mechanically dissociated using a potter and filtered with 180 µm and 50 µm mesh. Finally, cells were centrifuged, counted and diluted at 200.000 cells/ml.

Larvae of recipient batches were injected at 20, 30, 60 and 90 days post hatching (dph). 11 batches were injected with spermatogonia and 3 batches were injected with ovogonia (only at 30 dph). Recipient larvae were anesthetized with tricaine and approximately 5000 cells including GSC were injected into the peritoneal cavity of each recipient under binocular magnifier using a CellTrams microinjector and a Leica micromanipulator. Some of the injected cells floating in the abdominal cavity were expected to randomly attach to the abdominal wall. Injected larvae (200 larvae per group) were transferred into a recirculated system and left to recover for 3 days, then reared following the standard protocols.

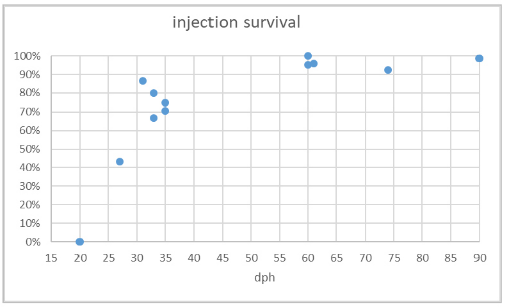

Fig 1. Survival rates after injection. Survivals recorded three days after injection of the GSC.

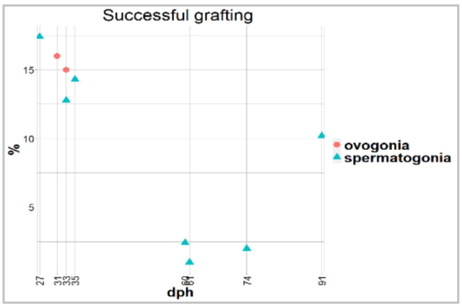

Fig 2. Successful GSC grafting rates. GSC grafting determined by flow cytometry at 1 year.

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To determine the best age for the injection, the survival rate was followed during the first three days following the injection and the success rate of GSC grafting was determined at 1 year. 100 fish of each group were sacrificed at that time to compare the ploidy levels of their gonads with that of a blood sample, using flow cytometry analyses. Gonads and fins of fish showing different ploidy levels were genotyped for thirteen microsatellite loci in order to find different allele contributions between the recipient's somatic tissue and the gonads. At 3 years the remaining recipient fish kept in maturing condition were sacrificed to observe their gonads. The gonads showing development or non-linear development were analysed using flow cytometry to compare their ploidy levels to that of the fins.

Results and Discussion

Post-injection survival rates were low at 27 dph (43%) but increased up to 100% at 90dph (668 mg) as shown in figure 1. Analysis of the survival rates after injection suggests that sea bass larvae are too small and too fragile to be injected before 30 dph (15 mg).

Our data also shows that the percentage of successfully grafted recipients varied from 1 to 17% with the best grafting success obtained for the recipient larvae injected from 27 to 35 dph (fig. 2). Interestingly, no difference was observed between testicular and ovarian cells grafting success at 30 dph.

Further investigations will include reproduction of injected recipients fish to control the viability and the functionality of the gametes derived from the grafted germ stem cells.

Our study confirms that GSC grafting is possible in sea bass despite the impossibility to inject newly hatched larvae. This biotechnology opens new possibilities for innovative applications aimed to: (i) conserve and regenerate the whole genetic characteristics (mitochondrial and nuclear genomes) of original and/or selected populations (Lareyre et al 2018), (ii) generate viable double haploids, (iii) create monosex populations.

Acknowledgement

This work was partially financially supported by Biogerm project (n°1700769) funded by the French Government and European Maritime Fisheries and Aquaculture Fund (call "Innovative Aquaculture 2017" managed by the FranceAgrimer Office.

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IDENTIFICATION OF NOVEL AND CONSERVED miRNAS IN PIKEPERCH (*Sander lucioperca*)

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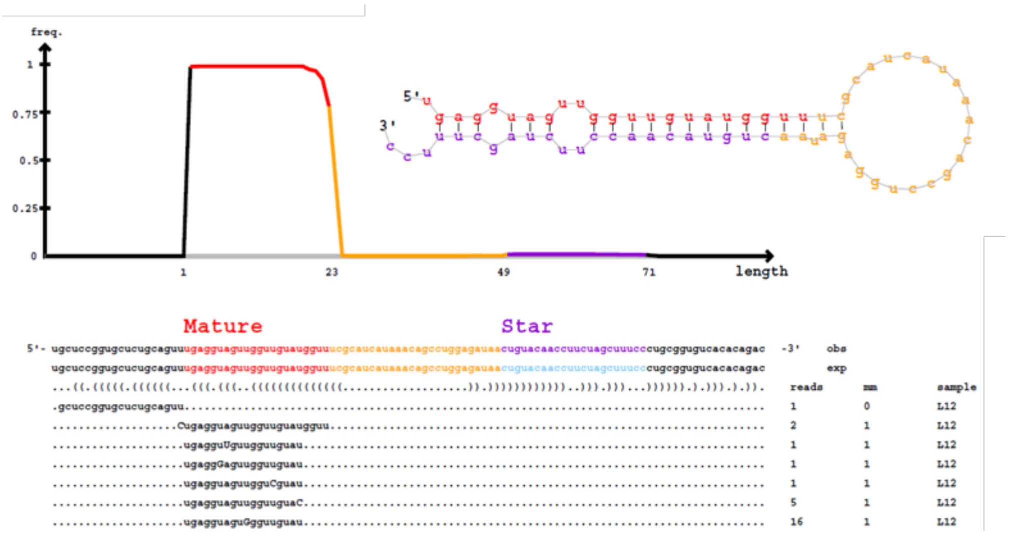
Introduction

Pikeperch is increasingly considered for commercial farming in recirculation aquaculture systems (RAS) in European aquaculture. In addition to its growing commercial importance, research on the genetic and genomic composition of the percid fish has also gained momentum. With the recent publications on the genome [1] and transcriptome [2], systematic exploration of functional genomics is feasible, especially in the context of relevant commercial traits. Here, the identification and characterization of regulatory elements like micro RNAs (miRNAs) represents another important part of the puzzle to explore, understand and improve the full scope of the performance of economically important traits in this fish species. Micro RNAs are short non-coding RNA molecules that play an important role in the transcriptional and post-transcriptional regulation of their target transcripts. They are involved in the regulation of multiple developmental and physiological processes like growth, the immune system, or stress response [3]. Data on miRNAs in teleost fish are still limited, with only 16 out of the over 26,000 species present in miRBase (05, April 2022; http://www.mirbase.org/). So far, there is no available information on miRNAs in pikeperch. We have identified and characterized the miRNA repertoire of pikeperch using deep sequencing and miRDeep analysis. Furthermore, we investigated specific expression patterns of the miRNAs in different tissues and developmental stages of this percid fish.

Material & Methods

Juvenile pikeperch stages, fingerlings and adult individuals used in this study were bred and reared in RAS system at the State Research Centre for Agriculture and Fisheries Mecklenburg-Vorpommern (Hohen Wangelin, Germany). A total of 20 samples including pools of eyed eggs, yolk sac larvae (2dph and 15 dph), and larvae (26dph) as well as spleen of two fingerling stages (3g and 35g) and liver, gills, head kidney, and spleen of adult fish were used for deep sequencing. An additional of 20 samples (a total of 40 samples) were used for real-time qPCR expression analysis.



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    ....................ugagguaguugguuguaC......................................................................   5       1       L12
    ....................ugagguaguGgguuguau......................................................................   16      1       L12
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Fig.1 Exemplary output from the miRDeep analyses for one identified miRNA precusor. The figure shows a graphical illustration of the hairpin-loop structure and the sequence information for the identified precursor. The mature 5p (red) and mature 3p (purple) miR as well as details on the counts of reads mapped to the precursor sequence are given.

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Total RNA was isolated from the samples by use of the mirVana miRNA isolation kit (Invitrogen), followed by Library preparation according to the manufacturer protocol using only RNA samples with RIN values ≥ 7 and the Illumina TruSeq Small RNA Library Preparation Kit (Illumina). RNA libraries were sequenced on the Illumina Genome Analyzer IIx sequencing platform. High quality reads were used for miRNA identification applying the miRDeep2 software package. The present version of the pikeperch genome assembly (GeneBank accession number: GCA_008315115.2) was used as reference genome. Predicted miRNA precursors with a miRDeep2 score ≥ 1.0e+2 were used in the downstream analysis, including annotation and estimation of tissue specific miRNA expression, as described elsewhere [4]. Real-time qPCR expression analysis of a set of predicted highly expressed miRNAs was performed by using the Biomark HD system (Fluidigm).

Results

Initial miRDeep analysis identified a total of 240 pikeperch miRNA genes with 375 corresponding 5p and 3p mature miRNAs in the different developmental stages of pikeperch meeting the threshold for the miRDeep score mentioned above (Fig.1). Of these, we could classify 234 as orthologs to evolutionary conserved miRNAs while seven are potential novel miRNAs. Furthermore, expression analysis revealed miRNA specific expression pattern across investigated developmental stages and tissues.

Discussion & Conclusions

In order to investigate and improve economically important traits in pikeperch for optimising its rearing conditions, it is necessary to gain basic knowledge of the genetic and genomic composition of this species. Our study provides the miRNA repertoire in pikeperch and supplies their specific expression patterns. Among these are miRNAs that may be of particular interest to the aquaculture industry as they are regulating economical important processes like metabolism, growth and resilience.

This work was funded by the European Maritime and Fisheries Fund (EMFF), the Ministry of Agriculture and the Environment in Mecklenburg-Western Pomerania (grants: MV-II.1-RN-001, MV-II.1-LM-001) and the research council of Norway (grant number 280839/E40).

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NURSERY CULTURE OF *Crassostrea gigas* IN RAS

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Introduction

Traditional flow-through oyster hatcheries and nurseries generally depend on local water sources, which limits the amount of suitable locations, and increases the risk for unwanted organisms and disease outbreaks. A closed recirculating aquaculture system (RAS) for the production of early stage bivalves has been reported, but literature is scarce. A RAS provides a controlled environment that minimizes water consumption, increases waste management, mitigates the summer mortality syndrome and is the ideal setting to produce specific-pathogen-free oysters. A RAS also enables production in regions where natural seed cannot be collected or where (clean) seawater is scarce. This project developed an indoor RAS for oyster spat. Animal growth was evaluated and compared to a closed, static system (batch). This study is the first to report growth of cupped oyster spat in a RAS.

Materials & Methods

Two trials of five weeks each, were carried out using nursery-stage Pacific oyster (*Crassostrea gigas*). All spat was obtained from a local oyster farm, De Oesterput bvba (Ostend, Belgium). Growth experiments were performed using different initial sizes and feeding regimes (table 1). One and two RASs were used in the first and second experiment, respectively. Each RAS held 500 L of filtered natural seawater, and included a drum filter, protein skimmer, header tank and three rearing tanks of 90 L each. The flow rate was 38 L h^{-1} per rearing tank, or ten times the tank volume per day. A batch system with 100% renewal rate every second day, was used as control and consisted of three rearing tanks of 90 L. Each rearing tank held one PVC cylinder basket, with a depth of 80 mm and a bottom mesh with 1 mm mesh size, that held 35 grams of spat. The water temperature was set at 19°C. All rearing tanks received a percentage of the oyster wet weight in dry weight live microalgae at a 1:1:1 composition of *Tetraselmis chuii*, *Chaetoceros muelleri* and *Isochrysis galbana* (table 1). All microalgae were obtained from Proviron (Hemiksem, Belgium). Total oyster biomass was kept constant by removing the grown biomass weekly. Shell length (SL), individual wet (WW) and dry weight (DW), as well as individual dry shell (SW) and dry tissue weight (TW) were measured weekly, and the specific growth rate (SGR) was calculated. Total ammonia nitrogen (TAN), calcium (Ca) and carbonate (CO_3) concentrations were monitored multiple times a week.

Results

1. Oyster growth

Overall, the results from the spat weight and length measurements indicate no or only a small difference between animals grown in batch and those grown in a RAS receiving 1.50% DW microalgae. An example can be seen in figure 1. In the first experiment, the SGR for WW is 44% and 41% per week for spat grown in the batch system and the RAS respectively, representing almost a doubling in total weight every two weeks.

2. Water quality variables

TAN concentrations were low throughout both experiments, never exceeding the value of 0.5 mg L^{-1}. A drop in CO_3 was observed in the RAS in both experiments, more so than for Ca (figure 2). Ca and CO_3 occupy around 95% of the shell composition, hence the drop in these substances is in line with the (relative fast) oyster spat shell growth. This observation is rarely mentioned in literature on bivalves in closed systems, and requires more in-depth investigation on the amount, the frequency and the form in which Ca and CO_3 consumption should be compensated for in a RAS for bivalves.

Table 1: treatments and initial spat length and weights for both trials.

Trial	System	DW microalgae (%)	Initial SL (mm)	Initial SW (mg)	Initial TW (mg)
1	RAS	1.50	4.05	6.2	1.4
	Batch	1.50			
2	RAS 1	1.50	2.64	2.1	0.4
	RAS 2	0.75			
	Batch	1.50			

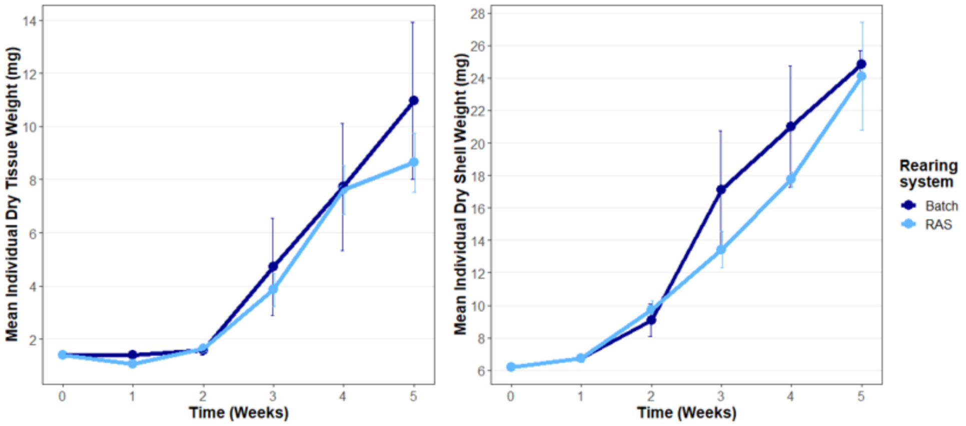

Figure 1: results from the first trial show that TW (left) and SW (right) increase at a similar trend in both rearing systems.

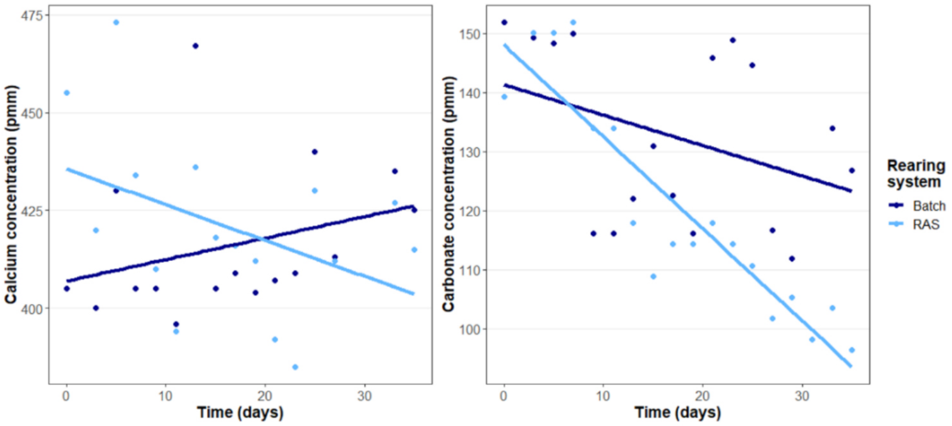

Figure 2: results from the first trial on the change of calcium (left) and carbonate (right) concentrations over time.

Conclusion

The results from the growth measurements demonstrate that a RAS environment is suitable for growing oyster spat. Oyster shell mineral requirements require more investigation to finetune Ca and CO_3 addition to RAS. Overall, the results so far indicate that a RAS could be a valuable alternative for future bivalve nurseries.

Challenges and opportunities

In further experiments, we will investigate the compensation for carbonate and calcium, as well as the potential for rearing the larval stadia and other oyster species (*e.g*. the European flat oyster, *Ostrea edulis*) in a RAS.

Acknowledgements

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IMPROVING MINERAL AND VITAMIN NUTRITION IN SENEGALESE SOLE

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Introduction

The production of high quality and healthy fish larvae and juveniles is an important target for the successful and competitive growth of the aquaculture industry. Still, most marine fish larvae produced under intensive rearing conditions are known to be affected by several afflictions, including low survival rates and a high incidence of skeletal anomalies. Amongst other factors, nutrition has a key role in skeletogenesis. While taking advantage of an industrial setting, we performed the nutritional optimization of a commercial-type larval feed in terms of vitamins and minerals, that can be potentially be used as a tool to mitigate skeletal anomalies relevant for fish welfare and production.

Materials and methods

A series of larval fish nutritional trials were performed, using as base feed formulas, commercial larval fish feed products available from SPAROS Lda. Several nutritional feed component modifications were performed: Ca:P ratio; Inorganic Vs. Organic mineral forms; Dietary levels of Mn and Zn; Selected B-Vitamin supplementation; Choline removal from premix. Growth (Dry weight, standard length, relative growth rates (RGR)), survival, economic (feed conversion ratio (FCR)) and bone status (Figure 1) criteria were assessed.

Results

The omission of calcium supplementation, that can have a potential antagonist effect on the bioavailability of other minerals and/or nutrients, from the premix used in larval feed lead to the observation that a low Ca:P ratio, ranging between 0.5 and 0.7, is adequate to support a high survival and growth performance in Senegalese sole post-larvae, without compromising bone development and skeletal formation.

The replacement of inorganic mineral forms (sulphates) of Cu, Fe, Mn and Zn by their organic glycinate-chelated forms, did not generate marked beneficial effects on somatic growth and bone development in Senegalese sole post-larvae fed high quality commercial microdiets.

Dietary levels of Mn (90 $mg.kg^{-1}$) and Zn (130 $mg.kg^{-1}$) could help improve larval survival, decrease the severity of vertebral malformations, and increase the deposition of Mn in bone.

B-Vitamin supplementation (B1, B6 and B12) to larval feeds brings no clear advantage in attempting to enhance the growth performance and bone status of Senegalese sole larvae.

Although choline supplementation to vitamin premixes can decrease their stability, it is still necessary when feeding Senegalese sole larvae as, it's removal can reduce growth criteria.

Conclusion

The conclusions obtained throughout this work demonstrate that mineral and vitamin levels and/or forms in commercial formulas are adequate for proper growth and bone development. Still, there is room for further nutritional optimization of inert microdiets when the potential beneficial effects of beyond requirement levels are considered and their impact on bone, immune and stress coping status of early-stage marine fish.

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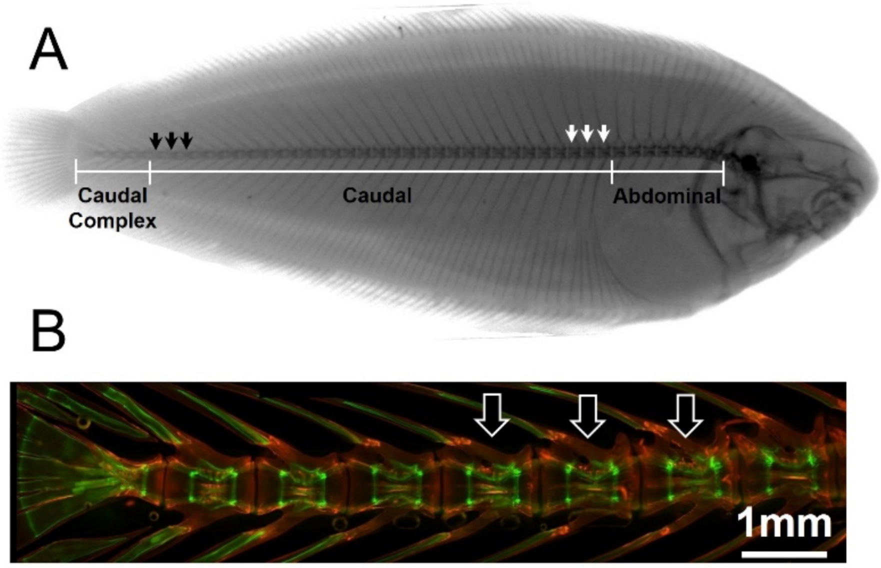

Figure 1- Bone status parameters. A-Microradiograph of Senegalese sole representing different vertebral areas (white lines) used in malformation analysis, vertebrae used for density analysis (white arrows) and vertebrae used for mineral apposition rate (MAR) analysis (black arrows). B- Fluorescence micrograph of vertebrae stained with Alizarin red-S (red) and Calcein (green). Black arrows indicate exemplative vertebrae used for MAR analysis. Bar is 1mm.

SOLID-STATE FERMENTED PLANT-BASED DIETS FOR EUROPEAN SEA BASS *Dicentrarchus labrax*: COMPLEMENTING TRIAL INTERPRETATION WITH A NUTRIENT-BASED MODEL

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Introduction

As the aquaculture industry continues to grow, aquafeed production must rely on alternative ingredients to fishmeal, which is unsustainable both from an environmental and economic perspective. Plant ingredients are a major alternative dietary protein source, however, dietary plant-based proteins present some constraints, such as high fiber content and anti-nutritional factors, which can limit nutrient digestibility (Sinha et al., 2011). Some biological processes can be applied to improve the nutritional quality of plant feedstuffs, such as Solid-State Fermentation (SSF). SSF is a cost-effective and eco-friendly biotechnological process that relies on fungi to digest the substrate, producing fungal biomass and increasing nutrient bioavailability (Soccol et al., 2017). Applying mathematical models, *a posteriori* to a feeding trial can be useful to assist the interpretation of the results, allowing to infer whether the observed performance is fully explained by the nutritional or environmental factors taken into account by the models or if there are unknown factors worthy of being explored that might have contributed to the results.

Materials and methods

This work explored the effects of SSF plant-based diets on the zootechnical performance of European sea bass (*Dicentrarchus labrax*). For that purpose, a mixture of soybean, rice bran, sunflower seed, and rapeseed meal (25% each) was fermented with *Aspergillus niger*. Then, four isoproteic (42%) and isolipidic (18%) diets were formulated including a non-fermented mixture and the SSF mixture at 20% and 40% (diets 20M, 20SFF, 40M, and 40SSF, respectively; Table 1). Triplicate groups of 20 fish, with an initial body weight of 10±0.02g, were fed for 50 days at 21°C in a recirculating system (RAS). After the *in vivo* trial, the same experimental conditions recorded throughout the trial were used to run simulations with the FEEDNETICS^{TM} nutrient-based model (SPAROS, Lda.). The main objective was to evaluate whether the fish performance observed in the *in vivo* trial is well predicted by the model, in order to infer whether there are unknown nutritional factors worthy of being explored in future research.

Results and Discussion

The *in vivo* growth performance was higher in fish fed with the 20M, 20SSF, and 40M diets, while feed efficiency was higher in fish fed with the 20M and 20SSF diets and significantly lower in fish fed with the 40SSF diet (Table 2). A clear suppression of feed intake was observed for the SFF diets, however, due to probably better nutrient availability, the 20SSF diet resulted in a relatively good growth performance, which is also reflected in high feed efficiency. The same was not observed for the 40SSF diet.

Overall, FEEDNETICS^{TM} predicts similar performance as it was observed in the *in vivo* trial, except for the dietary treatment 40SSF where the deviation between the simulated and observed values (here estimated as the MAPE) is higher compared to the other treatments (Figure 1). In this case, the model overestimates the observed growth. This leads us to infer that the reduced performance of the 40SFF diet is probably not fully explained by a decrease in diet palatability. There must be another factor that contributed to this loss of performance, that is affecting nutrient availability and/or utilization. The plausibility of this hypothesis is also supported by a reduced feed efficiency observed at the end of the *in vivo* trial.

Conclusion

In overall terms, the diets with the SFF mixture induced lower growth performance and feed intake on European sea bass. However, a positive effect on feed efficiency was observed for inclusion levels of 20% SFF. In turn, inclusion levels of 40% SFF did not show to improve feed efficiency, and the model results suggest that a factor other than the loss of palatability may have contributed to the loss of performance. Future research on SFF mixtures should focus on trying to compensate the loss of palatability and on identifying the causes related to the loss of performance when higher inclusion levels of SFF mixtures are considered.

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Table 1 – Proximate composition of the experimental diets.

	20M	20SSF	40M	40SSF
Dry matter (DM) (%)	96.8	90.8	96.3	90.4
Crude protein (CP) (% DM)	42.3	41.4	41.0	41.4
Crude lipids (CL) (% DM)	17.3	17.4	17.4	17.0
Ash (% DM)	8.1	9.3	9.4	9.8
Gross energy (GE) (KJ/g DM)	22.9	22.0	23.4	22.9

Table 2 – Growth performance of European sea bass fed with the experimental diets, at 21°C, for 50 days.

Diets	20M	20SSF	40M	40SSF	SEM	2-way ANOVA		
						F	I	F * I
Final body weight (FBW, g)	25,36 a	23,83 a	21,63 Ab	15,95 Bb	1,12	**	***	*
Weight gain (g kg ABW^{-1} day^{-1})	17,72 a	16,68 a	14,96 Ab	9,26 Bb	1,03	**	***	*
Feed Intake (g kg ABW^{-1} day^{-1})	18,98 A	15,91 a	17,38 B	12,22 b	0,79	***	**	ns
Feed efficiency (FE)	0,93 A	1,05 A	0,86 B	0,76 B	0,04	ns	**	ns

Values are presented as mean (n=3). SEM: pooled standard error of the mean.
Two-way ANOVA: Fermentation (F), Inclusion (I). non-significant (ns) ($P \geq 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

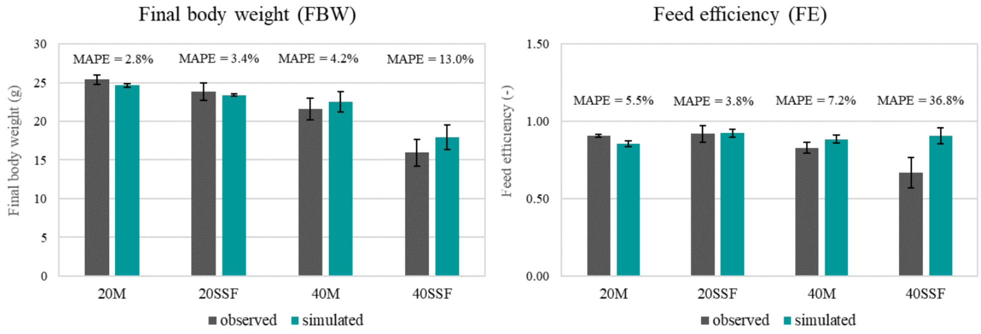

Figure 1 – Comparison between final body weight (FBW) and feed efficiency (FE) observed in the *in vivo* trial and simulated by FEEDNETICS^{TM}, where the deviation between the values is given by the mean absolute percentage error (MAPE).

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SOLID-STATE FERMENTED PLANT-FEEDSTUFF MIXTURE WITH *Aspergillus niger* FOR EUROPEAN SEA BASS (*Dicentrarchus labrax)* JUVENILES EFFECTS ON OXIDATIVE STRESS, IMMUNE SYSTEM, AND MICROBIOTA

Vieira, L. *[1,2], Filipe, D. [1,2,3], Fontinha, F. [1,2], Martins, N. [1,2], Magalhães, R. [1,2], Serra, C. R. [1,2], Oliva-Teles, A. [1,2], Belo, I. [3], Peres, H. [1,2]

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Introduction

Aquafeeds production has been increasing as a result of the ongoing increase in aquaculture production. However, this industry faces several challenges, mainly regarding production sustainability. The inclusion of vegetable ingredients and agroindustrial by-products is an excellent option to reduce aquafeeds' carbon footprint and promote a circular economy. However, vegetable ingredients display some disadvantages, such as the presence of anti-nutritional factors, higher fiber content, and poor palatability. Some biotechnological processes have been applied to this type of ingredient to mitigate these constraints. Solid-state Fermentation (SSF) is an eco-friendly and cheap biotechnological process in which fungi digest the substrate and reduce the presence of anti-nutritional compounds, enhancing the nutritional value of SSF products. Moreover, these secondary metabolites, antioxidants or β-glucans, may enhance fish's immune innate system. As it follows, this study aims to evaluate the oxidative stress status, inflammatory and metabolic gene expression, and gut microbiota modulation in European sea bass juveniles fed diets with a SSF plant-feedstuff mixture inclusion.

Materials and methods

A plant-based mixture composed of soybean, rice bran, sunflower, and rapeseed (25% each) was fermented with *Aspergillus niger* 2088 CECT for 7 days at 25 °C to obtain mycoprotein enriched biomass and enzyme production. Four isoproteic (42%) and isolipidic (18%) diets were formulated to contain 20% and 40% of the unfermented (Mix) and fermented mixture (SSF Mix): 20 Mix, 20 Mix SSF, 40 Mix, and 40 Mix SSF, respectively. A growth trial was conducted for 50 days in a recirculating water system, with European sea bass juveniles (*Dicentrarchus labrax*; initial body weight of 10g). The experimental diets were randomly assigned to triplicate groups of fish. At the end of the trial, three fish from each tank were randomly sampled after 4 hours of the first meal, and liver, intestine, and muscle were collected for oxidative stress and lipid peroxidation analysis, and liver and intestine for gene expression by RT-PCR. Another two fish from each tank were sampled at the same time, and intestinal mucosa and digesta were collected for posterior PCR-DGGE (denaturing gradient gel electrophoresis) fingerprints obtention.

Results

In the intestine, catalase (CAT), glutathione reductase (GR), and glucose 6-phosphate dehydrogenase (G6PDH) activities were unaffected by the inclusion level, nor by SSF. Glutathione peroxidase (GPX) was higher in fish fed the 40% mix diets. SSF increased superoxide dismutase (SOD) activity in fish fed the 20% SSF diet. Concerning the inclusion effect, SOD activity of fish fed the 40 Mix SSF was lower than 20 Mix SSF. Liver glutamate dehydrogenase (GDH), and aspartate aminotransferase (ASAT) activities were not affected by the diet composition. Alanine aminotransferase (ALAT) activity was higher in fish fed with 20% Mix diets. Liver CAT activity was higher in fish fed the 40% Mix diets, while GR and G6PDH were lower in fish fed the fermented diets, irrespective of the Mix inclusion level.

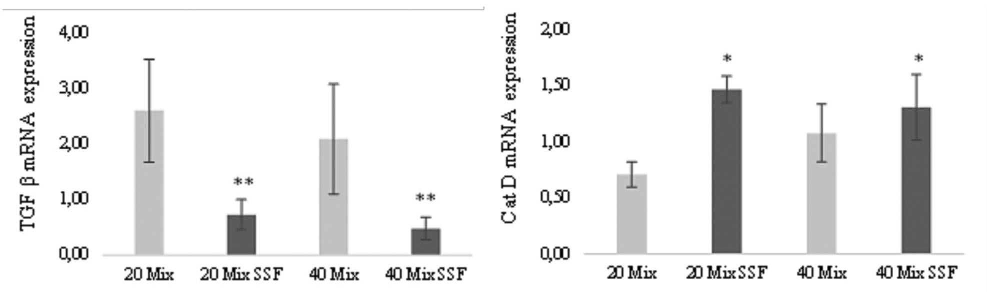

Fig. 1 - Quantitative expression of gut's transforming growth factor beta (TGF β), and liver's cathepsin D (Cat D) of European sea bass juveniles fed dietary treatments

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Values are presented as mean ± SEM (n=9). SEM: pooled standard error of the mean. A Two-way ANOVA was used to analyze the effect of inclusion level and SSF. The * indicates significant differences between SSF and non-SSF diets ($P \leq 0.05$) ; *$P < 0.05$; **$P < 0.01$.

Hepatic SOD activity was higher in fish fed the 40 Mix SSF diet compared to the 20 Mix SSF diet. Concerning the SSF effect, SOD activity increased when fish were fed the 20 Mix SSF compared to fish fed the 20 Mix diet. The intestine, liver, and muscle lipid peroxidation (LPO) were not affected by dietary treatments.

Caspase 3, interleukin 8, interleukin 10, tumor necrosis factor α, and insulin-like growth factor 1 gene expression was not affected by the diet composition. Transforming growth factor β gene expression was lower, while cathepsin D expression was higher in the SSF diets (Fig. 1).

PCR-DGGE fingerprints of fish fed the experimental diets showed that SSF increased the average intestinal number of operational taxonomic units (OTUs), microbial richness, and diversity. This was confirmed by the analysis of the sequenced gel bands that showed the appearance of numerous *Bacillus* spp. in fish fed with the SSF diets. The inclusion level increased microbial diversity when fish were fed the 40% vegetable mixture diets.

Conclusion

Overall, oxidative stress and lipid peroxidation of the intestine, liver, and muscle were unaffected by dietary treatments. SSF appears to have immunostimulant properties, due to the decrease of TGF when fish were fed the SSF diets. SSF of plant feedstuffs mixture had beneficial modulatory effects on intestinal microbiota, by promoting the abundance of *Bacillus* spp., which may produce antifungal compounds and enzymes, and may help fungal digestion in fish's gastrointestinal tract.

Acknowledgments

This work was funded by the Ocean3R project - Reduce pressures, restore and regenerate the NW-Portuguese ocean and waters, (ref NORTE-01-0145-FEDER-000064) within the research line "Decarbonising our waters and ocean", founded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF). This research was also supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

NUTRITIONAL EVALUATION OF DIETS INCLUDING A SOLID-STATE FERMENTED PLANT-FEEDSTUFF MIXTURE WITH *Aspergillus niger* FOR EUROPEAN SEA BASS (*Dicentrarchus labrax)* JUVENILES

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Introduction

Modern aquaculture relies on plant feedstuffs as the main alternative to unsustainable and expensive ingredients, such as fishmeal. Fish meal is the most complete ingredient for fish nutrition, lacks anti-nutritional factors, is highly digestible, and has a balanced amino acid profile, but it is no longer used as the main ingredient due to its unsustainability. On the other hand, plant feedstuffs are cheaper ingredients but present some constraints such as poor palatability, high fiber content, imbalanced amino acid profiles, and anti-nutritional factors. These limit aquafeeds' digestibility, feed efficiency, and overall fish growth performance. Biotechnological processes, such as Solid-state Fermentation (SSF), may be used to improve the nutritional properties of plant feedstuffs. SSF is a cheap and eco-friendly process that relies on fungi to digest the substrate, thus increasing nutrient bioavailability by producing fiber-degrading enzymes and fungal biomass. This study aims to evaluate the nutritional effects of including a solid-state fermented plant-feedstuff mixture in aquafeeds for European sea bass juveniles.

Materials and methods

A plant-based mixture (Mix: soybean, rice bran, sunflower, rapeseed; 25% each) was fermented with *Aspergillus niger* 2088 CECT (Colección Española de Cultivos Tipo) for 7 days at 25 °C to obtain mycoprotein enriched biomass, and carbohydrase production. Four isoproteic (42%) and isolipidic (18%) diets were formulated to include the fermented and non-fermented mixture at 20% and 40% (diets 20SSF, 20M, 40SSF, and 40M, respectively). A growth trial was performed in a recirculating water system (RAS) at 21 °C. Triplicate groups of 20 European sea bass juveniles (*Dicentrarchus labrax*; initial body weight of 10g) were fed each experimental diet for 50 days. At the end of the trial, fish's growth performance, feed intake, and feed efficiency were evaluated. Three fish from each tank were randomly sampled after 5 hours of the first meal, and the intestine was collected for digestive lipase, amylase, and total alkaline proteases activity determination. Blood was collected from the caudal vein with heparinized syringes, centrifuged at 10000 rpm for 10 minutes, and plasma was collected for total protein, glucose, cholesterol, and triglycerides determination.

Results

During the trial, mortality was low and was increased by dietary SSF. Irrespective of SSF, the growth performance of fish fed with the 40 Mix diets was lower than with 20 Mix diets. Regarding the SSF effect, growth performance of fish fed with 40 SSF was lower than that of the respective unfermented diet. Feed intake decreased with SSF and was lower when fish were fed the 40 Mix and 40 Mix SSF, compared to the 20% inclusion level. Feed efficiency, and protein efficiency ratio were significantly increased with the fermented inclusion in 20 Mix SSF and 40 Mix SSF. Nitrogen intake decreased when fish were fed the 20 Mix and 40 Mix fermented diets. Nitrogen retention decreased within the 40% inclusion level when fish were fed the 40 Mix SSF diet. Irrespective of the fermentation, nitrogen retention of fish fed with a 40 Mix SSF diet was lower than with 20 SSF (Table 1). Whole-body ash and lipid contents were unaffected by dietary SSF. Regarding the inclusion level, whole-body dry matter content increased when fish were fed a 20% Mix diet, rather than at 40%. Irrespective of the inclusion level, fish fed the 20 Mix diet had higher dry matter than 20 Mix SSF. Dietary SSF increased whole-body protein in the 20 Mix SSF diet and decreased in the 40 Mix SSF diet. Gross energy content was affected by the inclusion level, being higher when fish were fed the 20% inclusion diets. Amylase activity decreased when fish were fed the 20 Mix diet compared to the 40 Mix diet, irrespective of the SSF. Concerning the SSF effect, among the 40% inclusion level, fish fed the 40 Mix SSF had lower amylase activity compared to the respective non SSF one. Lipase activity increased when fish were fed the 20 Mix SSF, regardless of the inclusion level. Regarding the inclusion level, fish fed the 40 Mix diet had higher activity than the 20 Mix diet. Total alkaline proteases decreased when fish were fed with 20% inclusion level diets, irrespective of SSF. Regarding the SSF effect, fish fed with non-fermented diets had higher activity of proteases. Plasma metabolites cholesterol, total protein, and triglycerides were unaffected by dietary treatments. Regardless of the inclusion level, serum glucose was higher in the 40 Mix SSF diet compared to the respective unfermented one.

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Conclusion

SSF reduced overall growth performance in 40% inclusion and was not affected in 20SSF. Feed intake decreased in 20SSF, but feed efficiency increased. Protein efficiency ratio increased in 20SSF, which coincides with a higher whole-body protein deposition. Inclusion of 40SSF revealed a lower growth, feed efficiency, and protein retention. Plasma metabolites were not affected by dietary SSF. Overall, 20SSF reveals to be an interesting diet, allowing for more efficient use of the diet, reducing costs, and in a short feeding period without compromising fish's growth. Nevertheless, more studies are needed to explore the effects on growth performance of lower fermented inclusion levels, SSF with non-sporulating fungi, or to evaluate the potential of including extracts from this SSF mixture, to determine SSF's nutritional effects without fungal spores.

Acknowledgments

This work was funded by the Ocean3R project - Reduce pressures, restore and regenerate the NW-Portuguese ocean and waters, (ref NORTE-01-0145-FEDER-000064) within the research line "Decarbonising our waters and ocean", founded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF). This research was also supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

In vitro AND *in silico* EVALUATION OF THE PROBIOTIC POTENTIAL OF A *Lactococcus lactis* A12 STRAIN ISOLATED FROM A BACTERIAL COMPETITIVE EXCLUSION CULTURE DERIVED FROM NILE TILAPIA (*Oreochromis niloticus*) GUT MICROBIOTA

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This study aims to characterize the strain A12 isolated from a competitive exclusion culture derived from the intestinal contents of Nile tilapia (*Oreochromis niloticus*) and determine the probiotic potential regarding the antibacterial activity, hydrophobicity, and molecular identification and annotation using whole-genome sequencing and comparison to other isolates of the same species. The functional annotation of genes associated with probiotic activity was established. A12 showed antibacterial activity against *Streptococcus agalactiae* and *Aeromonas hydrophila*. Using the ANIb, ANIm, and Tetra indices (calculated in JSpeciesWS), A12 was identified as *Lactococcus lactis* A12. The pangenome analysis of Roary revealed that these bacteria possess unique genes that must be characterized in future research to determine if they are involved in adaptation to the fish environment. Annotation of the genome revealed the presence of essential genes involved in the biosynthesis of amino acids, vitamins, bacteriocin-like proteins, adhesion genes, and carbohydrate metabolism. Consequently, *in vitro* and *in silico* evaluation of the probiotic potential revealed a promising opportunity to use the selected A12 as probiotics in tilapia culture; however, in vivo experiments at various production scales are required.

PILOTING OF A SOFTWARE TOOL DESIGNED TO CALCULATE THE ENVIRONMENTAL PERFORMANCE OF MEDITERRANEAN AQUACULTURE PRODUCTS

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Introduction

Fish represents a significant part of the global food provision, accounting the 20% of the animal protein consumed by humans (Carvalho & Guillen, 2021). Since the past few decades, aquaculture has been subject to a significant production growth, which is reflected in a rise of the global aquaculture production of 527% from 1990 to 2018 (FAO, 2020). In 2019 there was a production of over 2 million fish tones in the Mediterranean from which 43% was aquaculture production (FAO, 2020).

In 2013 the European Commission (EC) proposes the Product Environmental Footprint (PEF) method as a common way of measuring environmental performance (COM2013/179/EU). This method establishes the Life Cycle Assessment (LCA) methodology to measure environmental performance of products represented by 16 environmental impact categories. Within this framework in 2018 the LIFE+ AQUAPEF project was launched with the aim to facilitate the environmental footprint calculation of Mediterranean aquaculture products. An important outcome of this project is the development of a software tool specifically designed to help the Mediterranean aquaculture sector to calculate the environmental performance of their products, complying PEF methodology, in specific the Product Environmental Footprint Category Rules (PEFCR) for marine fish. The study presented in this manuscript, describes the piloting stage of the software tool in several seabass and seabream producer companies within the Mediterranean aquaculture sector. Results allows to identify benefits and challenges of using the software tool in a regular basis within the aquaculture sector.

Approach and Methodology

In order to validate the efficiency and usefulness of the software tool, it was tested by several companies from different countries (Greece, Italy, Spain and Cyprus) of the Mediterranean aquaculture sector.

The tool transforms company specific data, gathered by the user, into 16 environmental impact categories recommended by the International Reference Life Cycle Data system (Fazio et al., 2018). This process is carried out based on secondary data coming from public databases that are EF-compliant tendered by the EC. Before loading data into the tool, the user needs to collect the information that will conform the inventory data to be used by the tool to perform the LCA. To facilitate this data collection, the user was provided with questionaries that ask for the specific information that should be collected in the tool. The software tool was developed following the methodology described in de Marine Fish PEFCR. It works via web; hence, the user can access to it in any computer with internet connection only using its username and password. The tool gathers the information of the main steps of the Mediterranean offshore aquaculture sector: feed production, juvenile production, growing in open sea-cages, preparation, and distribution. Moreover, it also considers retailer stage, consumption and end-of-life based on average data. Results obtained from the tool are based on the stablished functional unit that is 1 kg of edible fish product and covers the life cycle of the product from “cradle to gate”.

Workshops and training sessions were carried out to present the tool to the companies and to transfer them the minimum knowledge required to use the tool. Besides those activities, the companies were allowed to test the tool by their own during a period, after which more feedback were received.

Results

The companies in general shown great interest on the tool and found it useful for their activities. Furthermore, companies communicated their feedback for the tool, where the next topics were identified: i) the ability to cover all the activities of the value chain including external suppliers while protecting sensible information shown to be useful, ii) validation of the methodology used, iii) visibility of the methods and databases that are used for the calculations, and iv) tool being translated to different languages used within the European Mediterranean was highly appreciated. Feedback related to tool usability, specifically about tool design, information content and how this information is shown and/or distributed within the user interface were also communicated.

Other subjects like the validity of the tool when other species than seabass and seabream are intended to be studied, and how the social and economic impacts can be included in the tool, arise during the piloting stage.

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Conclusion

Based on the feedback received from the companies that participated on the tool piloting, three main topics were addressed: i) sensible data confidentiality and security, which was ensured by the appropriate protocols from the server provider and by giving specific access permissions to each participant of the value chain; ii) training required, even though the tool user-interface was developed in a "friendly" way to allow the use of it by any member of the company, the experience after piloting the tool in a "real-life situation" suggest that the ideal user must possess some knowledge of LCA and domine the company operations related to fish production, this topic was addressed by including resources in the tool like video tutorials and info-boxes and by organizing training sessions with the companies; and iii) tool certification, companies are concern about the possibility of being required by the EU to present a valid PEF report in the future, reason why the need of a certified tool becomes important.

To validate the tool, not only in the production of seabass and seabream in the Mediterranean aquaculture sector, but also for its usefulness when dealing with other species and aquaculture areas outside the Mediterranean, the replicability stage is currently being carried out. During this stage the tool is being tested with producers of salmon, and croaker, located in Atlantic waters like the north of Spain and Norway.

Acknowledge

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PERFORMANCE CHARACTERIZATION OF A DIFFUSED AERATION BASIN FOR CARBON DIOXIDE REMOVAL IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Fish produce carbon dioxide (CO_2) as a normal outcome of aerobic metabolism and excrete this gas through their gills into the surrounding water. In recirculating aquaculture systems (RAS) with high stocking densities, dissolved CO_2 accumulates to unsafe levels unless some form of carbon dioxide control is used. Properly designed CO_2 control unit processes are needed to maintain safe levels of dissolved CO_2 in RAS culture tanks at high stocking densities. Typical CO_2 control unit processes include force-ventilated columns with or without packing media, shallow diffused aeration basins, and passive ventilation trickling biofilters. Force-ventilated packed columns can remove 40% to 60% of dissolved CO_2 from RAS water when properly designed. However, pumping is required to transport water to the top of force-ventilated packed columns. Pumps require capital investment, 24/7/365 operating costs, routine maintenance, and have a relatively short depreciation period. Therefore, it is critical to maximize CO_2 removal efficiency to minimize the water flow that must be pumped when using a force-ventilated packed column. Alternatively, unit processes that remove CO_2 using less energy can be utilized if design criteria are established. The objective of this project was to characterize the dissolved CO_2 removal performance of a low head or gravity flow CO_2 control technology and develop the associated design criteria for use in RAS mass balances and engineering.

Materials and methods

This project studied a diffused aeration basin that uses diffused air as the CO_2 stripping gas in a relatively shallow basin. Diffused aeration basins are often used in current RAS designs immediately after a moving bed biofilter. Water gravity flows from the moving bed biofilter into the diffused aeration basin with no water pumping required. This study evaluated diffused aeration basin hydraulic loadings (407, 611, 814, and 1222 lpm/m^2), influent CO_2 levels (10, 15, and 25 mg/L), and diffused air flow (G:L of 2 and 5) to characterize CO_2 removal efficiencies in a research-scale aeration basin that was 1.2 m by 0.9 m by 0.9 m deep (1 m^3). The project setup consisted of an influent tank using a low head oxygenator to inject CO_2 to achieve the desired influent dissolved CO_2 levels and a diffused aeration basin with pump and sump for returning treated water to the influent tank. OxyGuard International A/S portable CO_2 sensors monitored the dissolved CO_2 in the influent tank and after the diffused aeration basin as it returned to the low head oxygenator. Diffused air for aeration was provided by a regenerative blower and air manifold at the bottom of the aeration basin.

Results

The results of the study will be reported along with recommendations for optimizing the design of diffused aeration basins in RAS. Preliminary example results are shown in Figure 1.

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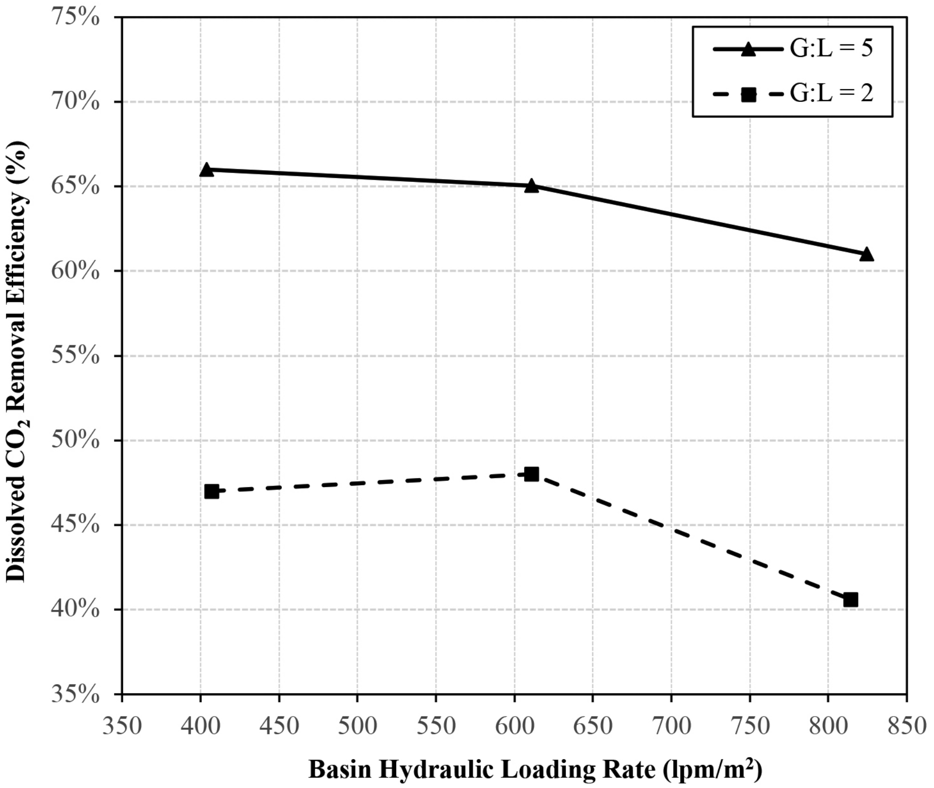

Figure 1. Dissolved CO_2 removal efficiency for a research-scale diffused aeration basin treating an inlet CO_2 concentration of 25 mg/L at a high air flow rate (G:L = 5) and a low air flow rate (G:L = 2).

EVALUATION OF A VELOCITY ASSIST INLET FOR IMPROVEMENT OF HYDRODYNAMICS IN COMMERCIAL-SCALE DUAL DRAIN FISH TANKS USED IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

One type of fish tank used in recirculating aquaculture systems (RAS) is the Cornell-style dual drain circular tank. Cornell-style dual drain fish tanks have two drains: a bottom center drain, and an elevated sidewall drain. Cornell-style dual-drain tanks are advantageous because the rotational velocity caused by the inlet water injection creates a self-cleaning effect. In operation, a Cornell-style dual drain tank functions as a swirl separator with waste solids collecting at the tank center and flowing out the bottom center drain. These fish tanks also provide a relatively uniform environment for the fish, with minimal gradients in dissolved oxygen (Davidson & Summerfelt, 2004).

In a typical Cornell-style dual drain circular tank, the injection of treated RAS water causes a rotational velocity that is highest at the tank perimeter and decreases toward the tank center. Recent research indicates increased water velocities provide performance benefits for Atlantic salmon post-smolts raised in fish tanks (Timmerhaus et al., 2021). To achieve these benefits, an additional inlet can be installed on separate pumped loop that removes water from the fish tank and re-injects it to provide increased water velocities. This internally pumped "velocity assist" inlet aims to increase water velocities available to larger post-smolt and harvest-size salmon in commercial-scale Cornell-style dual drain circular fish tanks. This study sought to evaluate the effect of the velocity assist technique by testing combinations of three different water flow rates and three different nozzle head loss configurations for a velocity assist inlet in a semi-commercial scale RAS used for land-based growout of Atlantic salmon with the intent of determining optimal combinations for increasing water velocities at minimum energy.

Materials and methods

The velocity assist technique was empirically tested by the addition of a pumped velocity assist loop with adjustable inlet in a 150 m^3 Cornell-style dual drain circular tank that is part of the semi-commercial scale RAS at the Freshwater Institute (USA). Empirical water velocity sampling of the currents in the tank were carried out for all combinations of the following: ~30% of the main flow as velocity assist flow (1136 lpm), ~50% of the main flow as velocity assist flow (2271 lpm), ~60% of the main flow as velocity assist flow (2763 lpm) and low (0.14 bar), medium (0.33 bar), high (0.56 bar) velocity assist inlet nozzle headloss. Water flow was adjusted by changing the number of pumps operating and adjusting a control valve. Nozzle headloss was adjusted by changing the number of 2.54cm openings on the inlet. Water velocity measurements were collected using a SonTek Argonaut-ADV 3-axis Doppler velocity meter at three depths across two cross-sections once steady state conditions were achieved for each combination of flow and headloss (Figure 1). Water velocities were also characterized for operation of the tank without the velocity assist inlet in operation.

Results

Preliminary results indicate that operation of the velocity assist inlet improved velocity profiles in the 150 m^3 Cornell-style dual drain circular tank over the base case without a velocity assist inlet. Higher velocities were observed throughout the tank with higher velocity assist inlet water flow and nozzle headloss. The complete results of the evaluation will be presented along with recommendations for optimizing the velocity assist technique in commercial-scale fish tanks for RAS.

Conclusions

The use of a velocity assist inlet effectively decouples the control of tank water velocities for improved fish welfare from the primary inlet flow and allows for more control as fish increase in size.

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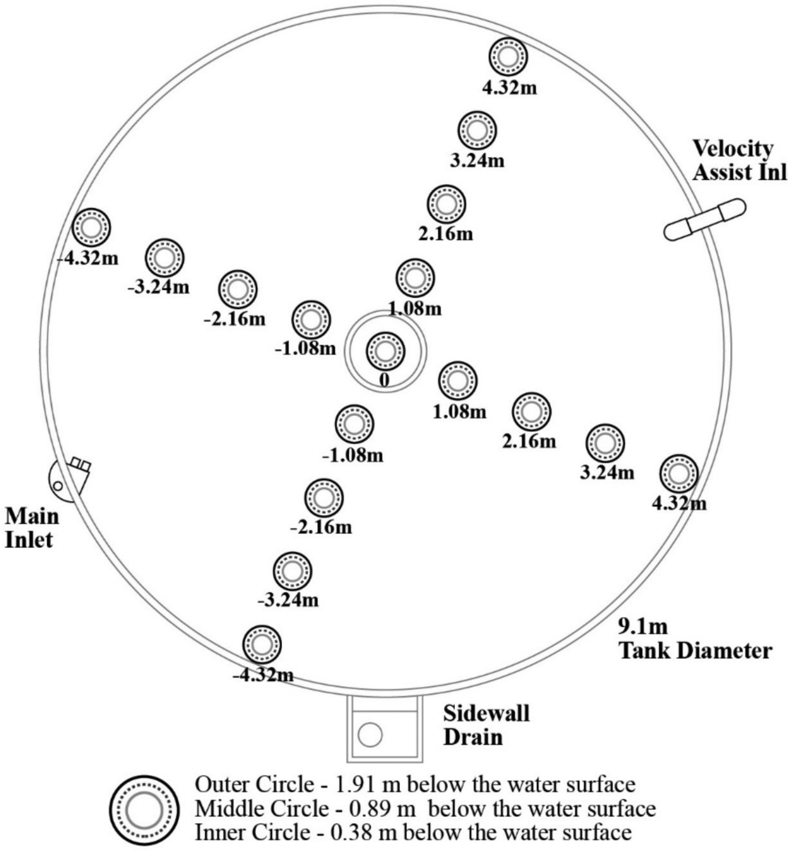

Figure 1. Locations of water velocity measurements in the 150 m^3 Cornell-style dual drain circular tank that is part of the semi-commercial RAS at the Freshwater Institute facility (USA).

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THE EFFICACY OF VARIOUS CHEMICALS IN THE DISINFECTION OF DUSKY KOB (*Argyrosomus japonicus*) EGGS AND HATCHERY WATER

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Introduction

Dusky kob (*Argyrosomus japonicus*) has been identified as a promising food fish in the South African marine finfish industry. To support industry growth, existing hatcheries frequently sell or donate excess eggs to neighbouring farms, raising concern over the potential transfer of infectious disease agents. Chemical disinfectants are frequently used to prevent transmission of diseases, but their application has shown large variations between finfish species and very little is known about the efficacy and impact of disinfectants on dusky kob eggs or larvae. Bacterial diseases are particularly prevalent in finfish farming, constituting about 55% of infectious disease outbreaks compared with viruses, parasites and fungi. Since little is known about the microorganisms, particularly bacteria, associated with dusky kob and their rearing systems in South Africa, this study aimed to assess the efficacy of various chemicals in disinfecting dusky kob eggs and the rearing water and their subsequent impact on larval development.

Materials and methods

Microbial samples were collected from dusky kob eggs and hatchery water from Pure Ocean (Pty) Ltd, a commercial dusky kob farm in the East London Industrial Development Zone (ELIDZ) of South Africa. Dusky kob eggs and hatchery rearing water were exposed to four different chemical disinfectants for 5 min at difference levels: povo-iodine at 30 mg/L (PI30); formaldehyde at 2000 mg/L (F2000); chloramine-T at 25 mg/L (CT25); and hydrogen peroxide at 200 mg/L (HP200), and compared with a control where no disinfectant was added. Following treatment, eggs samples were rinsed with autoclaved seawater, water samples filtered to concentrate bacteria, the eggs were homogenized and then tenfold serial dilutions of both concentrated bacteria and egg samples were plated on a general media commonly used for growth of marine bacteria (TSA) and a *Vibrio* spp. selective media (TCBS).

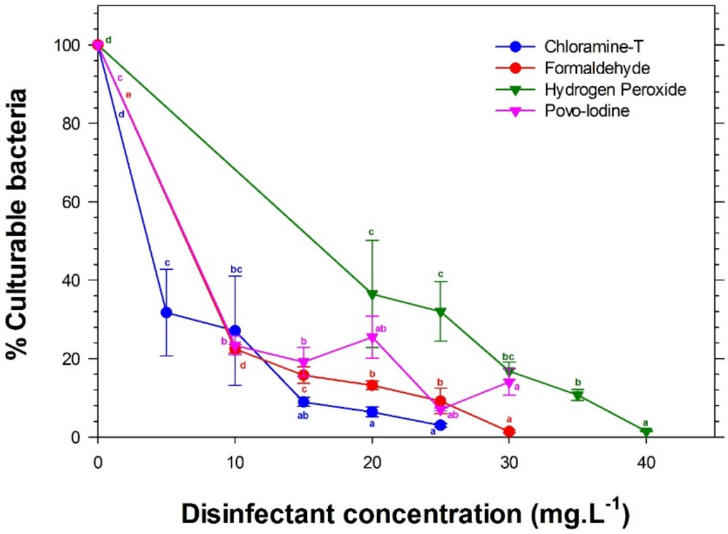

Fig 1: Effect of varying the concentration of each chemical disinfectant (a. Chloramine-T; b. formaldehyde; c. hydrogen peroxide; d. povo-iodine) on the number of culturable bacteria in seawater.

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Results

All chemical disinfectants tested in this study were able to reduce the number of culturable bacteria associated with hatchery rearing water and on dusky kob eggs. Treatments with povo-iodine at 30 mg/L (PI30); and formaldehyde at 2000 mg/L (F2000) significantly decreased bacterial growth on TSA media by 43 and 88%, respectively, while chloramine-T at 25 mg/L (CT25); and hydrogen peroxide at 200 mg/L (HP200) only decreased bacterial growth by 3 and 10%, respectively. Similarly, PI30 and F2000 decreased the growth of *Vibrio* species by 79 and 75%, respectively, while CT25 and HP200 decreased *Vibrio* spp. by only 12 and 14%, respectively (Figure 1). None of the chemical disinfectant appeared to have any adverse effects on larval development as no significant differences were recorded in any of the larval development parameters of dusky kob between any of the treatments and the control (no disinfectant).

Discussion

This study showed that all tested disinfectant agents were capable of reducing the culturability of bacteria in seawater in a dose dependent manner. However, povo-iodine (30 mg/L) and formalin (2000 mg/L) were shown to be more effective at reducing the number of bacteria associated with dusky kob eggs following a 5 minute exposure period. None of the tested disinfectants had an adverse effect on early larval development, suggesting that these disinfectants have potential for treatment and control of bacteria associated with seawater and eggs in dusky kob hatcheries.

THE WELFARE OF TAGGING: THE EFFECT OF CHRONIC STRESS ON WOUND HEALING AND WELFARE IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

To provide fish a voice to communicate feelings, needs and worries within its population requires the use of technology that can analyse and interpret multiple forms of data which can be processed into actions for providing better welfare. This can be done with new age tagging technology, however, the tagging process can not cause welfare to be compromised if this form of communication is to be valid. This study aims to document how chronic stress is visible through primary, secondary, and tertiary stress responses and their effects on the visible wound healing cascade and fish welfare.

Materials and methods

266 Atlantic salmon (*salmo salar*) were distributed into 9 indoor tanks (1.0 m^{-3}) with continuous flow of seawater with salinity 33.5‰, temperature of 7.3 ± 0.3 °C, and oxygen level of 97.7%. 24 hour light regime, with dry feed dispensed automatically in excess was provided during the experiment and acclimation phase lasting 68 days was present. Three experimental groups (in triplicate, total of nine tanks) were used: (1) Control, (2) Wound, and (3) Wound + Stress. Sampling was done once per group every week starting from week 0 (pre-stress) to week 8. The control group had no manipulation done, the wound group had a dummy tag surgically implanted into the abdominal cavity, and the wound + stress group had the same surgical implant as the wound group while also being subject to a daily crowding stressor by lowering the water in their tank to increase density from 30 kg/m3 to 315 kg/m^3 lasting roughly 30 minutes. Primary and secondary stress responses were measured from the blood plasma including CRH, ACTH, dopamine, and cortisol from the primary and glucose, lactate, magnesium, calcium, chloride, and osmolality from the secondary. Tertiary stress responses such as weight, length and fin erosion of 5 different fins were used.

Results

Primary stress responses with most significance are shown in Fig.1, secondary stress responses had few significant differences between the three groups with no conclusion drawn. For the fin erosion the wound + stress group suffered significant erosion in fins after week 3. Using the wound + stress group to rank severity of fin damage, the order of most damaged at week 8 compared to week 1 is as follows, pectoral, pelvic, anal, caudal, dorsal. There was positive correlation between the aggregated fin score of the pelvic, pectoral, and anal regions and plasma in wound + stress group (r_s= 0,700). No such correlation was shown in the control (r_s= -0,714) and wound (r_s= -0.407) group. While for the wound healing (Fig.2) the inflammation of the wound in the wound + stress group was larger and slower healing than the wound group. Also the inside wound was larger and healed slower in the wound + stress group than the wound group.

Discussion & Conclusion

The present experiment studies the effect tagged Atlantic salmon experience during chronic stress, and how the fish respond to daily crowding stress in terms of allostatic response, overall welfare and visible wound healing. The main observations from the study are: (1) Wounded individuals do not suffer from chronic stress; (2) Wound + stressed individuals suffer from allostatic overload type 2 with an increase of ACTH starting at week 4 to an increase in baseline levels of plasma starting at week 6; (3) Fin erosion is significant in stressed fish where damage to fins occurs well before the allostatic overload, indicating a possible use of fin erosion as a pre-indicator of stressful conditions; (4) Stressed individuals suffer from a larger inflammation period in weeks 1-2 while also their inside wounds heals slower than unstressed wounded fish, suggesting a neuroimmunoendocrine regulatory feedback; (5) Choice of suture can result in better welfare for tagging studies; (6) Internal wound healing is slower in stressed fish and plays a key role in understanding tag retention. (7) Unstressed fish do not suffer compromised welfare when tagging is introduced and they are allowed to recover, while tagging with daily stress compromises welfare, indicating the need for good aquaculture practices to be used post tagging to establish a healing opportunity.

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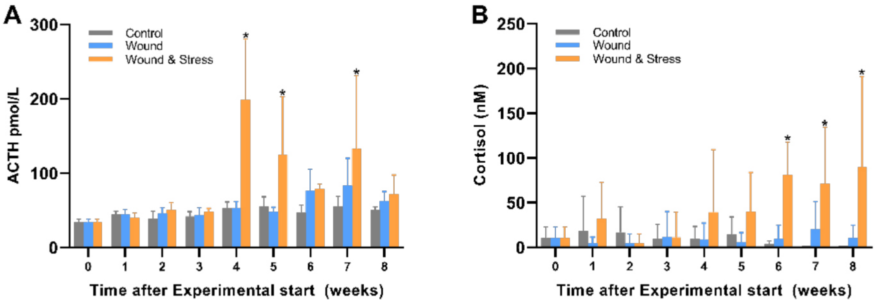

Fig.1 A) Plasma ACTH for all three groups, B) Plasma cortisol for all three groups. * above bars represents statistical difference to both groups (P ≤ 0.05).

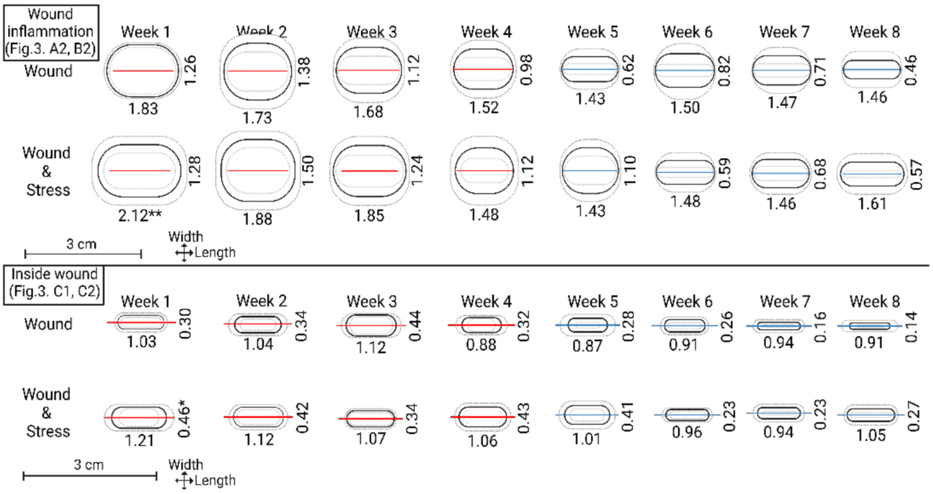

Fig.2 Top section: Wound inflammation width and length measurements for each week represented as ovals with standard deviation in dashed ovals. Line within ovals represents mean wound length (A1 Fig.3.). A red line indicates not all replicates within that week had wound width (B1 Fig.3.) = 0.0cm, a blue line indicates all replicates within that week had wound width = 0.0cm. Lower sections: Inside wound healing width and length, represented the same as the top section. Significance between groups represented as * = P ≤ 0.05, ** = P ≤ 0.01.

SUSTAINABLE EXPLOITATION OF FARMED LUMPFISH – FOOD NEOPHOBIA'S INFLUENCE ON INTRODUCING NEW FISH SPICIES IN A NEW MARKET

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Introduction

The challenges with sea lice in the salmon aquaculture industry have resulted in various amounts of measures to remove lice from the salmon in the net pens. Among the measures taken, the use of cleaner fish placed together with the salmon to eat lice from the salmon, have become an effective way to reduce the amount lice. The increased popularity of using cleanerfish (lumpfish and different wrasse species) have further resulted in a new industry, farming of cleanerfish. The production of lumpfish for this purpose has grown, significantly the last years, from just above 400 thousand fish in 2012 to nearly 36 million in 2020 (Source: Directorate of Fisheries).

A challenge with the use of lumpfish for eating lice is that they stop eating lice when they reach about 200-300 grams. The industry has however paid limited attention to the after-use of this fish, meaning that millions of fish goes to waste or in best case are used for ensilage when taken out from the salmon pens. Our aim was to find a more sustainable after-use for this resource. The most sustainable solution for the fish was in our opinion to.

Our aim is to find a suitable market for human consumption of farmed lumpfish. Challenging task as introducing new products in itself is challenging, and this fish have in addition a history that may cause even more challenge. The fact that the lumpfish have been used to eat lice for salmon in early life stage can be problematic for some consumers.

Theoretical background

The current trends towards globalization and cultural diversity have led to an increased demand for non-traditional food not only in Western countries but also in homogenized Asian societies. However, people vary considerably in their willingness to try new foods, with some individuals showing great pleasure in eating new foods and others showing strong aversions to them (Ritchey et al., 2003).

Introduction of new food are in itself a challenging task, and introducing new food to a new market, may be even more challenging. Food neophobia is defined as the unwillingness or refusal to eat or the tendency to avoid new foods (Pliner and Hobden, 1992). This food neophobia results in high failure rates for innovative and novel food products (Barrena and Sanchez, 2012).

Food neophobia (FN) is generally characterized as a personality trait, a continuum along which people can be placed in terms of their tendency to accept or avoid new foods (Pliner and Salvy, 2006). Since FN can influence preferences toward novel foods (Tuorila et al., 2001), understanding its potential impact on consumers' food selections is an important issue for food product developers and marketers. Food neophobia can impact scores on product evaluations, particularly for novel products (Henriques, King, & Meiselman, 2009).

A systematic review of Food Neophobia studies the last 30 years (Rabadan and Bernabéu, 2021) showed that most studies were conducted in developed countries, mainly in the US and Europe. It is important to include several countries as differences have been reported between countries, such as between Koreans and American (Chung et al., 2012) and between well-educated and wealthy people from Western and emerging countries (Brunner and Nuttavuthisit, 2019). In emerging countries, this consumer segment was more conservative (more neophobic) about food, while in developed countries this segment showed lower levels of FN. According to Rabadan and Bernabéu (2021) specific attention should be paid to fast-growing countries in Asia, where society and food consumption are changing rapidly as results shows that FN in societies decreases with increased income, education, and urbanization (Koivistu and Sjöden, 1996; Meiselman et al., 2010; Predieri et al., 2020; Prescott et al., 2002; Beltrán et al., 2016; Schickenberg et al., 2008), Beyond economic development, the cultural background of a country seems crucial to explain the level of FN of its inhabitants (Rabadan and Bernabéu, 2021).

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FN is interesting to investigate as it has a negative effect on the willingness to try new or unfamiliar products (Fernández-Ruiz et al., 2013) which is essential when aiming for introducing a new seafood species to a new market. Olabi et al. (2009) indicate that it would be challenging for food companies to launch foods that are ‘‘highly novel non-traditional foods’’ into markets with high food neophobia levels and to assess whether these markets would require the elements of more aggressive marketing campaigns, such as the provision of a high number of free samples and free coupons (Olabi et al., 2009).

No research found investigating neophobia of industrial buyers, important gate-keepers…

Thus, our aim is to investigate FN of industrial buyers in South Korea.

Methodological approach
A pilot study conducted in Norway testing lumpfish in different milieus revealed a more positive perception of eating the fish in different Asian milieus. Thus, we chose two different Asian countries for further investigation: South Korea and Vietnam. Lumpfish were sent from Norway to Seoul and Nah Trang and distributed to different industrial buyers (number). They were given the task to evaluate the fish and make dishes they found suitable for the fish and their cultural cuisine. The product test was followed up with in-depth interviews asking questions about their perceptions about the fish; the appearance, texture, smell, size, and taste. We also asked about their perceptions regarding the fact that the fish have been used to eat sea lice from the salmon in the aquaculture industry. The interviews were conducted via teams and…

Results
The results of the product test and interviews revealed quite negative perceptions about the fish.
Lumpfish
Not for eating. Big trend in skin care for women – so maybe processed info collagen powder or somewhat
Maybe spicy stew. Separate skin because it’s too thick.
Should be filleted because of how it looks. Used for fritter dish.
”we have many uglier fishes, but this is different from ugly – its scary”
“it wont be easy to sell”
“I felt a bit uncomfortable. Actually I was very uncomfortable. I was doubtful if I can cook it deliciously.”
“It definitely didn’t have a mouthwatering look”
It did not make a favorable impression”
“To sell the lumpfish round, honestly, the skin color is not favorable and there are too much inedible part.”
“Delivering factual info to the consumers will be keen”
“It would take time to persuade people”
“need supporting evidence that it is not harmful to eat”

The name cleaner fish itself would give some sort of repulsion to people. Korean would never eat fish with this name. That doesn’t suit Koreans’ cultural sentiments. Even I as a chef feel uncomfortable as well.
“No one knew about Pantagonian toothfish before it got popular.”
“We are continuously looking for new fish and new dish.”
The amount of seafood catch in Korea keeps decreasing, I can see an opportunity for them. It is worth to try.
Positive: “Consumers are always looking for something new”
Opposite: “ Koreans are very conservative people. They don’t like unfamiliar things. They don’t even consider at all if thing are new and not familiar. It is extremely difficult to penetrate the market with a new item”

“In China it, it seems that things sell anyway. Koreans don’t like new items. They would check through the internet first, for example lumpfish, it they look at the picture of lumpfish on the internet, they would say “Nope, no way”
“Whomever the first penguin is, he must put a tremendous effort. Otherwise, its almost impossible.”

when entering into a market with a more neophobic cultural background, marketers may focus on how their non-traditional foods can be made to be more adaptable and familiar to local markets by using well-known food ingredients and familiar spices (Choe and Cho, 2011).

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Appendix A.

Annex Pliner and Hobden's (1992) Food Neofobia Scale (FNS)
(1) I am constantly sampling new and different foods (R).
(2) I don't trust new foods.
(3) If I don't know what is in the food, I won't try it.
(4) I like foods from different countries (R).
(5) Ethnic food looks too weird to eat.
(6) At dinner parties, I will try a new food (R).
(7) I am afraid to eat things I have never had before.
(8) I am very particular about foods I will eat.
(9) I will eat almost anything (R).
(10) I like to try new ethnic restaurants (R). (R) The positive items have been reversed.

THE ROLE OF TRACEABILITY AS AN ENABLER RESILIENCE, EFFICIENCY, AND SOCIAL AND ENVIRONMENTAL SUSTAINABILITY IN GLOBAL SHRIMP SUPPLY CHAINS

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Introduction

Consumer demand for seafood, including shrimp, has never been higher. Shrimp supply chains, both aquaculture and wild caught, are complex and opaque ecosystems of supply chain actors that bring product from many countries to consumers and restaurants around the world. Lack of transparency and data sharing across these actors creates a myriad of very serious problems for seafood companies, consumers, workers, and the environment, problems that are often created due to lack of investment in technology rather than the lack of available technology.

During the 20-year period from 2000 and 2020 the global shrimp market nearly doubled from 17.7B to $33.8B, an increase of 90%. Looking forward, by the year 2028 the global shrimp market is projected to grow another 60% to $53.63 billion in 2028 at a CAGR of 6.81%.

Market Dynamics

1. High demand (30 years in a row)
2. Declining *wild* shrimp and prawn populations due to overfishing
3. Large and growing number of farmed shrimp operators and *farmed* shrimp and prawn supply (55% of the shrimp on the market are from farmed operations versus 45% wild caught)
4. Significant consolidation and M&A activity of large, global, seafood companies (10 seafood companies represent 36% of the global production) with profit margins of 25%-35%.
5. Retailers with average profit margins of 35%-45%
6. Massive cost reduction pressure on downstream supply chain players (fishermen, processors, farmers) to support higher margins for large seafood companies and retailers who have the leverage to demand leaner financials
7. Not unlike food in general, the regulatory profile of the industry is relatively loose, particularly in the countries where much of the world's production comes from.

In this presentation I will specifically identify how granular and end-to-end traceability can impact positive change and performance in the following five areas.

1. Unsustainable Practices
2. Bycatch (Wild Caught)
3. Slave Labor (Wild Caught)
4. Overfishing (Wild Caught)
5. Mangrove habitat destruction (Farmed)
6. Overuse of antibiotics (Farmed)
7. Social Costs (Farmed)
8. Disease as a result of poorly circulating farm tanks (Farmed)
9. Slavery (Wild Caught & Farmed)
10. Waste
11. Mislabeling
12. Supply Chain Resilience
13. Operational Efficiency and costs/profit margins

I will also introduce the concept Social Contracts within global supply chains, and discuss the need for enhance interoperability across the systems of supply chain partners

SLEEPING WITH THE FISHES: USING BRAIN ACTIVITY TO ASSESS INDUCTION TIMES OF FIVE ANESTHETHICS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Assessing the induction of anesthesia often relies on visual observation of the animal[1]. However, evidence shows that visual indicators are not always reliable[1-3]. A more conservative method to assess insensibility is analysis of electroencephalogram (EEG) where visually evoked responses (VERs) from visual stimulus are recorded, and absence of these are considered a reliable sign of insensibility[3]. A recent study revealed an alarming mismatch between visual consciousness indicators and brain activity[2]. They showed that rainbow trout (*Oncorhynchus mykiss*) might perceive their surroundings for several minutes after being immobilized following CO_2- stunning, highlighting the importance of measuring brain activity when assessing sensibility[2]. Here, we apply a non-invasive method of acquiring brain activity data to identify the required dose of five anesthetics to render rainbow trout insensible[4].

Materials and methods

A silicone cup equipped with electrodes[1] was secured onto the head of lightly sedated rainbow trout (636.8 ± 16.1 g) to record EEG, and a light flashed twice per second to evoke visual responses. A wireless recording system[5] in the experimental tank and an overhead camera allowed non-invasive heart rate measurement and visual observation. Once the fish was calm and clear signals in the investigated variables were established, one of five anesthetics was added to the tank at 2-3 different dosages (see Fig. 1). Induction lasted for a maximum of 10 minutes or until VERs were abolished. After quick transfer to clean water, recovery lasted for a maximum of 15 minutes, or until observable consciousness and VERs were regained. Visual consciousness indicators were considered lost when the fish had fully lost equilibrium and swimming ability. The data for time until loss of VERs and visual consciousness indicators were analyzed using the Kaplan-Meier method and a log-rank (Mantel-Cox) test.

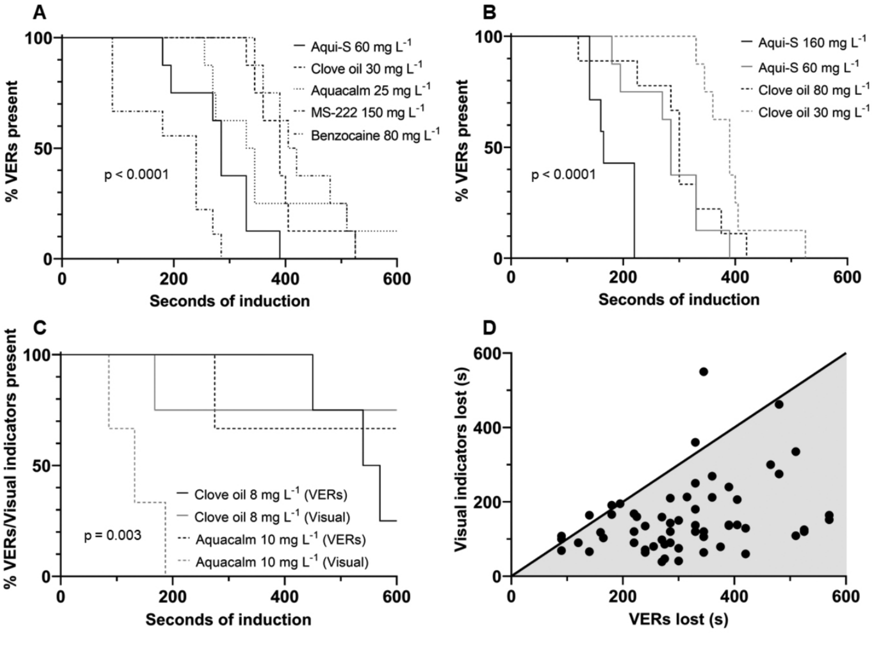

Fig. 1. Induction times of narcosis using five compounds, and the relationship between VERs and visual indicators. A. Time to loss of VERs with five commonly used compounds. B. Time to loss of VERs with different doses of Aqui-S and clove oil. C. Time to loss of VERs and visual indicators using clove oil and Aquacalm. D. Time to loss of VERs versus loss of visual consciousness indicators across groups. The black line illustrates a 1:1 relationship, and data points in the gray area represent a welfare hazard.

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Results and discussion

This is the first comprehensive evaluation of various common anesthetic compounds using non-invasively acquired brain activity data. We show that the induction to narcosis differs between compounds in terms of required time and dose, and that there is substantial individual variation (Fig. 1A-B). We further show a clear mismatch between the loss of VERs and visual consciousness indicators (Fig. 1C-D), and thus support the notion that basing assessment of anesthesia induction on visual indicators alone can be a welfare hazard[4]. Aquacalm showed the most pronounced mismatch (see Fig 1C). Our data further indicate that clove oil and Aqui-S may interfere with visual processing in rainbow trout. VERs were abolished in observably conscious individuals given clove oil, and some individuals anesthetized with clove oil or Aqui-S regained observable consciousness before VERs during recovery. This suspected sight loss represents a potential welfare hazard and indicates that VERs may not be suitable for assessing consciousness when using these compounds. All compounds caused cardio-ventilatory depression, which causes hypoxemia[6]. Benzocaine also occasionally induced involuntary defecation. In conclusion, we show that anesthetizing fish is associated with considerable welfare hazards and propose that species-specific evaluations based on appropriate methods are needed for developing reliable and ethically acceptable standards.

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GROWTH AND MINERALS COMPOSITION OF CULTIVATED SUGAR KELP IN THE NORTH OF NORWAY: SITE SELECTION, DEPTH AND SORI ORIGIN

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Introduction

In Norway, as in many parts of Europe, kelp cultivation is a relatively new industry. Interest in the cultivation of kelp is also increasing in the north of Norway. To cultivate kelp efficiently, understanding the physical and biological factors that affect survival and growth as well as developing appropriate production strategies will be among the keys to success. In the present study, *S. latissima* was cultivated at two sites (Kraknes and Rotsund), 90 km apart, in Troms, northern Norway (69-70 °N) in 2020. The effect of site, depth and sori origin (Kraknes and Rotsund) on *S. latissima* growth, biofouling, minerals and potentially toxic elements (PTEs) content was studied. This is the first study on mineral content of *S. latissima* cultivated in the Artic in Norway and the first investigation into the effect of sporeling translocation on the growth and quality of this species.

Materials and methods

S. latissima sporelings were incubated for 8 weeks in seawater in two flowthrough cylinders in the Nofima seaweed hatchery in Tromsø. Seawater temperature were kept at about 10°C in the first 6 weeks and then gradually decreased to ambient seawater temperature. When the sporelings reached an average length of approximately 0.5 cm they were deployed in at Kraknes and Rotsund sites. Environmental factors, growth, biofouling were monitored during the cultivation period from March to August. Major elements (calcium, potassium, magnesium, sodium and phosphorus), potential toxic elements (arsenic, cadmium, lead, mercury) and iodine contents of *S. latissima* were analyzed.

Results and Discussion

Cultivation sites had the strongest effect on the growth and quality of cultivated *S. latissima* even within a small geographical area. Large variations in the frond length and wet weight were observed between sites. The site with lower seawater temperature and no fresh-water influence had better growth and later outbreak of epibionts. Sori origin also had a significant effect on the growth, *S. latissima* produced from Kraknes sori had higher frond length and wet weight irrespective of grow-out site and grew best at their own site. This indicates inherent genetic difference in growth traits, as well as local adaptation to the natal environment for optimal growth.

The iodine content in general was high and increased with cultivation depth, and the results showed lower iodine content at the freshwater influenced site (Rotsund). The heavy metal content varied significantly between sites and sori origin and were lower than the recommended maximum level for food supplements in EU regulations.

The present study shows that both growth and mineral content vary profoundly within the same geographical region and it thereby underlines the importance of thorough site selection and using local traits with high growth rates for seeding and cultivation to achieve maximum biomass, higher quality and economic viability.

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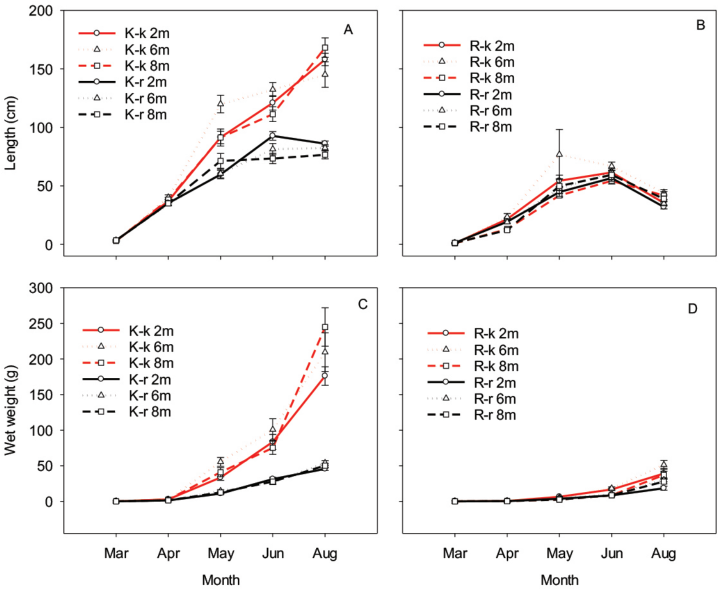

Fig.1 Frond length (cm) and wet weight (g) of cultivated *S. latissima* from two different sori origin at three depth intervals (0-2, 6-7 and 8-10 m) at both sites.

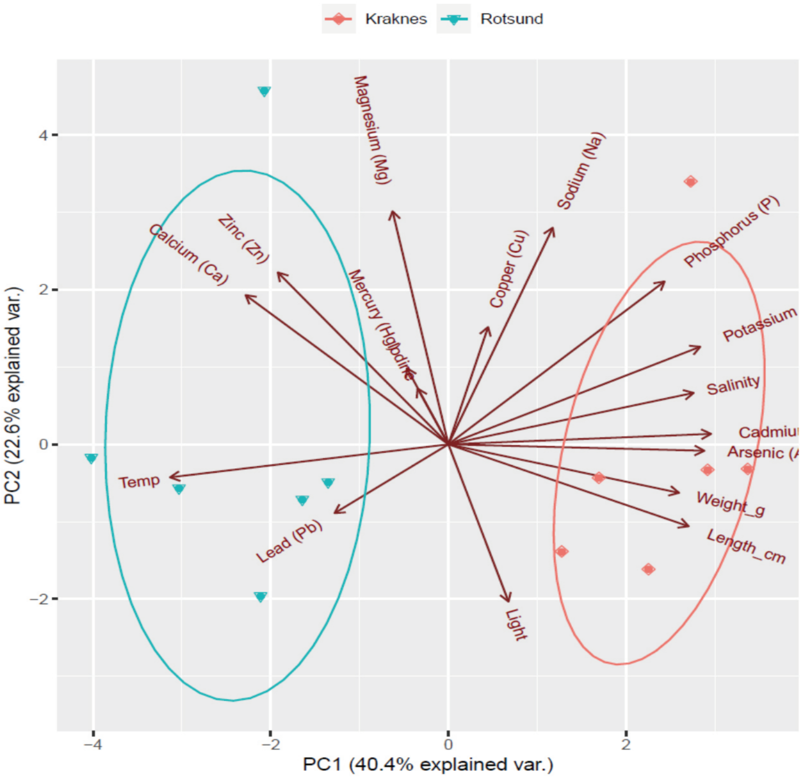

Fig2. Principal component analysis of length, wet weight, light, temperature and mineral content of *S. latissima* harvested in June at two cultivation sites (Kraknes and Rotsund).

SCHOOL CAMPAIGNS IN NIGERIA TO PROMOTE SUSTAINABLE AQUACULTURE AND OCEAN LITERACY

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The children and youth are the future of sustainable aquaculture and the ocean, in ASTRAL we recognise this hence an education campaign within the ASTRAL project is being developed and targeted at primary and secondary school students in Nigeria. This campaign aims to gather the importance of safe and nutritious aquaculture seafood also addressing climate change resilience and ocean influence. The campaign involves several schools across 3 states namely Lagos, Ogun and Oyo reaching a total of 450 students.

ASTRAL (All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture) is a Horizon 2020 project with the objective to promote Integrated Multi-Trophic Aquaculture (IMTA) farming system while increasing social awareness towards the consumption of sustainably produced seafood across the Atlantic countries. To target the young generation, the ASTRAL school campaign among other objectives aims at creating awareness and increase the knowledge base of children and youth on the following: the importance of safe and nutritious aquaculture seafood, IMTA, the benefit of the Atlantic Ocean and the Blue Economy, Climate change mitigation and resilience, ocean influence, aquatic pollution as well as the role and responsibility of the young generation on Ocean protection.

To achieve the ASTRAL goals, the objectives were communicated to different primary and secondary school teachers and students. These schools were adopted as ASTRAL schools forming ASTRAL School Clubs to make learning fun outside the regular curriculum objective of human capital development of the project. Some of the activities of these clubs are Do-it-yourself activities such as setting up small fish farms in the school and their homes(aquariums), creative drawing and video images, telling stories and essay writing on the subject matters. Other activities include educating on waste management (talks on pollution), awareness on recycling and upcycling (developing products from waste) and organizing clean-ups in schools, streets, parks and beaches.

There has been visitation by these schools on excursions to tour aquaculture farms and IMTA models in NIOMR. Researchers are also saddled with the responsibility of engaging these students in their schools.

ASTRAL Schools Clubs participated in the Children Day's 2022 Symposium at NIOMR. With 85 students and 21 teachers from 7 schools within Lagos metropolitan. There were talks from researchers on nutritious aquaculture seafood and the benefits, of care for the ocean.

The impact evaluation of these activities will be done through a competition among the schools on different occasions to achieve the ASTRAL aim. Further information on ASTRAL project can be found at www.astral-project.eu/

Figure 1. Schools on excursions to NIOMR to experience a practical view of various aspects of aquaculture production, the blue economy, climate change and ocean influence.

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Figure 2. NIOMR researchers at ASTRAL School club for its activities in Oyo state to the left, and Badagry, Lagos state to the right.

Figure 3. School Clubs participation in Children Day's 2022 Symposium at NIOMR.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 863034.

REPRODUCTIVE POTENTIAL AND POPULATION PERSISTENCE OF PACIFIC OYSTERS *Crassostrea (Magallana) gigas* IN A MACROTIDAL ESTUARY

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Introduction

While production of Pacific oysters *Crassostrea* (*Magallana*) *gigas* is approved across the UK under licence, currently there are only two oyster farming areas in Wales. Development of aquaculture is embedded in the objectives of the Welsh National Marine Plan, and national objectives for its sustainable expansion support economic development in rural coastal communities. However, potential colonisation of intertidal habitats by oysters, as seen along the south coast of England, is an issue of concern in coastal management and biodiversity conservation. Policy development regarding the future of Pacific oyster aquaculture is pending further information about the current status of *C. gigas* populations. Such information includes the extent to which reproduction of farmed oysters may act as a source of self-sustaining escaped (feral) populations across marine habitats, especially those comprising designated conservation features. The Welsh coast is an interesting case study for the potential for range extension of feral Pacific oysters, as environmental conditions may be marginal for successful reproduction and recruitment (and hence the risk of reproductive escape from aquaculture farms may be relatively low). In this study, we considered the potential for sustainable expansion of Pacific oyster aquaculture within the macrotidal estuary at Milford Haven (south Wales, UK).

Materials & Methods

Densities of intertidal *C. gigas* oysters in the Milford Haven waterway were re-assessed at five sites previously surveyed in 1998, using quadrat survey and timed searches. For each site, a Gunderson population model was used to estimate abundance of Pacific oysters, based on available area of suitable substrate for larval settlement (Phase 3 EUNIS biotope codes), with a correction factor for post-settlement mortality. The potential for recruitment from spawning events within Milford Haven was investigated using hydrodynamic- and larval dispersal modelling of potential transport and dispersal of larvae released from a range of locations, and under contrasting river flow scenarios. UK Meteorological Office AMM7 forecast model climate projections for past (1990-2018), present-day (August 2020 to August 2021) and future sea surface temperatures (2020-2099) were calibrated using monthly observed mean sea temperature data (1991-2007). The reproductive potential of oysters was assessed using a degree days formula, with thresholds for spawning and larval development of 600 and 825 cumulative degree days above 10.55°C, respectively (Mann, 1979).

Results & Discussion

The intertidal feral Pacific oyster population in the Milford Haven has not increased over the 23-year interval between surveys, at 3,209 (0.11 m^{-2}) and 2,949 (0.10 m^{-2}) in 1998 and 2021, respectively. This was despite dispersal modelling showing that oysters in the estuary could be self-recruiting to some extent, with relatively high retention of larvae within the estuary, originating from the more upstream release sites (Figure 1). We found that, given the well-mixed macrotidal conditions, seawater temperatures are only sufficient to support sporadic spawning events, with even more limited larval settlement. Sufficient degree days for maturation and spawning occurred in only 3 of the 18 years considered (1990-2018).

Maximum summer seawater temperature conditions may constrain successful settlement to fewer years than degree-day calculations, as peak temperatures rarely reach the threshold (>18-20°C) required to trigger spawning events. Low summer seawater temperatures would also be expected to support relatively low larval development and settlement success (Rico-Villa et al., 2009); in no past modelled years were total degree days sufficient for spawning and settlement. Future seawater temperatures in Milford Haven may become sufficient for more frequent spawning and settlement, although it is not possible to determine timescales for when these thresholds may be reached. While the prevailing conditions on the Welsh coast are currently at the limit for successful reproduction of Pacific oysters, projected future range expansion across NW Europe (King et al. 2021) and larval transport pathways from more southerly populations on the coasts SW England (Wood et al. 2021) may support recruitment to the south Wales coast, even while regional conditions still limit reproductive expansion.

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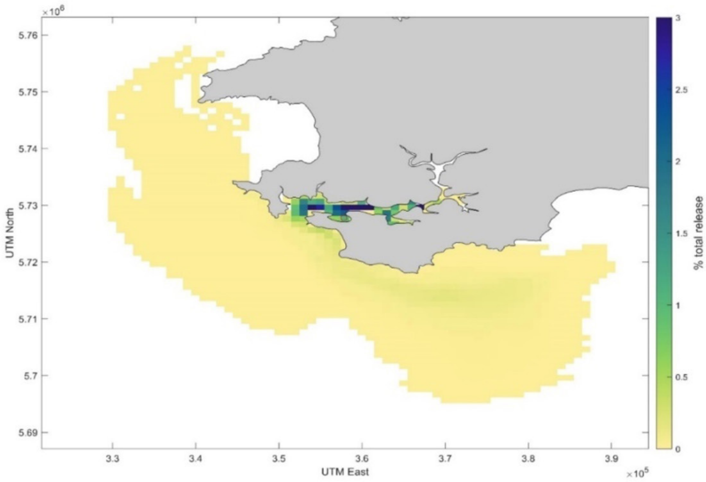

Figure 1 Simulated larval dispersal potential from the upper estuary in Milford Haven, showing the relative density of particle locations between 10 and 14 days from release.

Acknowledgements

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MARINE YEAST (*Candida sake*) CULTURED ON HERRING BRINE WASTE AS PROTEIN AND OMEGA-3 SOURCE FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Yeasts can convert organic side streams into high value protein and have become one of the most promising alternative feed ingredients for aquaculture and specifically salmonid fish (Agboola et al., 2021)extensive studies exist on the role of yeast cell wall components in modulating health responses of fish. However, research on its use as a major protein source in fish diets is still in its infancy. The current review collates, synthesises and discusses the prospects of five major yeast species as future protein ingredients with respect to their nutritional adequacy in fish. Nutritional quality of Saccharomyces cerevisiae, Cyberlindnera jadinii, Kluyveromyces marxianus, Blastobotrys adeninivorans and Wickerhamomyces anomalus and their use as replacement for fishmeal and soy protein in the diets of Atlantic salmon and rainbow trout are discussed based on three protein quality indices: chemical score, essential amino acid index and ideal protein concept based on the first limiting amino acids, methionine. The crude protein contents of yeast (40–55%. The range of side streams which may be used for yeast cultivation is broad and different yeast strains thrive in different culture conditions. *Saccharomyces cerevisiae* and most other terrestrial yeast do not tolerate high salt concentrations, rendering marine side streams unsuitable for cultivation of these yeasts. *Candida sake*, on the other hand, is a cold water marine yeast adapted to high salt concentrations and thus can utilize processing side streams of marine biomass. Similar to other yeast used as additives in feed, low levels of *C. sake* have been shown to have immune stimulating effects in the Indian prawn.

The aim of the present study was therefore to cultivate *C. sake* on herring brine (processing water of the herring industry) and to evaluate *C. sake* as a nutrient source and immunostimulant in feed for rainbow trout (*Oncorhynchus mykiss*).

The cultivation substrate was comprised of 50% pre-salting brine from North Sea herring and 10 % molasses. After freeze drying, a sub-sample of the product was exposed to an additional heat treatment (105°C). The experimental diets were formulated to substitute a fish meal-based control (C) diet with 20% *C. sake* (Y20), 20% heat-treated *C. sake* (YE20) or 3% *C. sake* (Y3). All diets were extruded, and fish were fed twice daily for 10 weeks. Measurements of weight and length were taken monthly. After finalizing the feeding trial, 61 fish from C and Y3 diet, were intraperitoneally injected with either LPS or PBS. Fish were sampled for tissue both at the end of the feeding trial and 12- and 24-hour post injection. The tissue samples included: blood, liver, head kidney, spleen, and intestinal mucosa. Additionally, samples of both proximal and distal intestine were used to assess intestinal nutrient uptake and barrier functions *in vitro*.

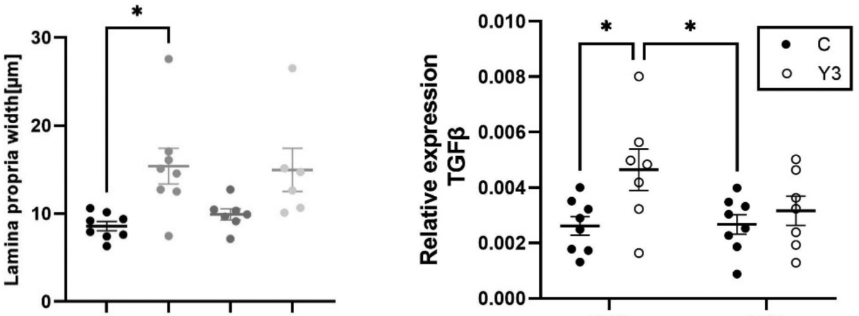

Fig 1. Left: Histological results; Average lamina propria width in distal intestine of juvenile rainbow trout fed either control (C), 3% *C. sake* (Y3), 20% *C. sake* (Y20) or 20% heat treated *C. sake* (YE20). *Right: qPCR results;* relative expression of TGFβ for rainbow trout injected with either PBS or LBS and fed either C or Y3 diet. * = p-values <0.05, bars = mean ± SE.

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Results

The *C. sake* product contained 54% protein and 13% fat including 1% DHA and 1.1% EPA. The results of the feeding trial revealed no effect on specific growth rate, feed conversion, condition factor and fat deposition in any of the diets. An increase in lamina propria width was observed in the distal intestine of fish fed the Y3 diet (Fig 1. left). Other parameters of intestinal health including other morphological measures and electrophysiology were unaffected by *C. sake* inclusion. Heat treating *C. sake* improved the apparent digestibility coefficient (ADC) of dry matter by 7% and gross energy (GE) by 2.7%, but reduced the overall amino acid ADC by ca. 3%. The ADC of *C. sake* for crude protein, crude fat, amino acids and GE was generally above 80%. LPS simulation had no effect on mRNA concentrations of selected immune genes, but increased levels of TGFβ in the 3% diet compared to the control fish were observed (Fig 1. right).

Discussion

The present study demonstrated the viability of cultivating *C. sake* on herring brine. Additional heat treatment of the yeast biomass increased dry matter and gross energy digestibility of the feed, possibly do to the rupture of long, for salmonids indigestible, polysaccharides (Agboola et al., 2021)extensive studies exist on the role of yeast cell wall components in modulating health responses of fish. However, research on its use as a major protein source in fish diets is still in its infancy. The current review collates, synthesises and discusses the prospects of five major yeast species as future protein ingredients with respect to their nutritional adequacy in fish. Nutritional quality of Saccharomyces cerevisiae, Cyberlindnera jadinii, Kluyveromyces marxianus, Blastobotrys adeninivorans and Wickerhamomyces anomalus and their use as replacement for fishmeal and soy protein in the diets of Atlantic salmon and rainbow trout are discussed based on three protein quality indices: chemical score, essential amino acid index and ideal protein concept based on the first limiting amino acids, methionine. The crude protein contents of yeast (40–55%. The amino acids content, and ADC however decreased after heat treatment indicating endothermic breakdown of amino acids. However, the heat treatment of 105°C is rather mild considering extrusion temperatures of generally above 100°C. Thus, other mechanisms causing the lower ADC may be involved. *C. sake* inclusion of 3% resulted in an increase in lamina propria width indicating immune stimulation and possibly infiltration of lymphocytes (Gisbert et al., 2013). While lamina propria width is a common histopathological indicator of intestinal inflammation (enteritis), no effect was observed in fish fed either diet with 20% yeast inclusion. This suggest that the increased lamina propria width most likely is not due to enteritis but rather a stimulation of the gut associated immune tissue. The 3% *C. sake* inclusion also increased transcription of TGFβ in the proximal intestine further suggesting an immune stimulating effect. This effect was LPS independent, and no other immune genes were affected.

Conclusion

The production of *Candida sake* using herring brine was successful and yielded a product containing over 50% protein and 3% omega-3 fatty acids. *C. sake* inclusion in feeds for rainbow trout did not affect growth and feed utilization. Due its nutrient profile and the sustainable production using industry process water, *C. sake* has great potential as an aquafeed ingredient. Downstream processing techniques increase the bioavailability of complex carbohydrate but alternative techniques, such as autolysis, may be more suitable than heat. Three percent yeast inclusion may alter gene expression and intestinal morphology. However, additional research is needed to understand the implications of these mild effects on innate immune response and disease resistance in rainbow trout.

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THE REUSE OF BIOFLOC MATURE WATER IN SHRIMP CULTURE OF *Litopenaeus vannamei* IN SUPERINTENSIVE BFT SYSTEM

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Introduction

In the biofloc technology culture system (BFT System) the water reuse, with a well-established microbial community where nitrification it is already taking place and in a presence of nitrate, is important to obtain a better stability of nitrogen compounds throughout a new production cycle. Krummenauer et al., (2014) reported that a minimum inoculum of 25% accelerated the formation of microbial aggregates in the BFT system. However, information on the minimum inoculum from the reuse of water rich in mature bioflocs is limited. The proposed of this study is evaluate the zootechnical performance and water quality parameters in treatments with different levels of inoculum (mg L^{-1}) in order to determine the minimum inoculum necessary for a rapid stabilization of nitrogen compounds in a new culture cycle of *L. vannamei*.

Material and Methods

The study lasted 45 days, until the nitrification cycle was completed in the control treatment with the detection of nitrate in the culture water. The experiment was carried out in 18 tanks with a volume of 400 liters, in six treatments, with different initial concentrations of total suspended solids (inoculum): 0 mg L^{-1}, 2.5 mg L^{-1}, 5 mg L^{-1}, 10 mg L^{-1}, 20 mg L^{-1} and 40 mg L^{-1}. During the experiment no water changes were made, unless the nitrite level exceeded 20 mg/L. In this case 30% of total volume was changed with previously chlorinated and de-chlorinated seawater. *L. vannamei* juveniles were stocked with an initial weight of 0.8g±0.1, at a density of 400 shrimp m^{-3}. The animals were fed twice a day at 08:00h and 17:00h with specific commercial ration containing 38% crude protein. The water with mature bioflocs, it means, that already had undergone the complete nitrification process until the appearance of nitrate, was collected from a raceway with *L. vannamei* culture in progress. Sugarcane molasses, with about 36% of carbon in its composition, was used as a source of organic carbon in the experimental units when the ammonia exceeded 1 mg L^{-1}.

Results

There was no significant difference between the physicochemical parameters of water, except for nitrogen. Ammonia and nitrite were higher in the control and lower in the treatments with the addition of inoculum, and nitrate showed an inverse pattern, it was lower in the control treatment and higher in the inoculum treatments (Figure 1).

More sugarcane molasses and water changes were used in the control treatment. The use of molasses was inversely proportional to the inoculum concentration, the higher the inoculum concentration, the lower the use of molasses. Regarding the zootechnical performance of shrimp, survival was lower in the control treatment, followed by the 2.5 mg L^{-1} treatment. In treatments 5, 10, 20 and 40 mg L^{-1}, survival ranged from 93 to 95%. Final weight was higher in treatments with lower survival and the opposite was observed for FCR (Feed conversion rate). Biomass and productivity were higher where inoculum was used, with better results in the 5 mg L^{-1} treatment.

Discussion

The use of a mature inoculum with a well-established nitrification process at the beginning of the culture was essential to avoid the oscillation of nitrogen compounds, as occurred in the control treatment without inoculum. This stabilization of nitrogen compounds directly reflected the better zootechnical performance of shrimp and the lower use of water and molasses to maintain water quality. Shrimp survival was low in the control treatment and 2.5, due to prolonged exposure to high concentrations of ammonia and nitrite that exceeded the safety level proposed by Lin & Chen (2001, 2003), of 3.95 mg L^{-1} and 25.7 mg L^{-1} respectively.

Conclusion

The results demonstrate that an inoculum concentration of 5 mg L^{-1} used in the starting of the culture of *L. vannamei* in a biofloc system, maintains water parameters more stable, uses fewer inputs of water and molasses and obtains a better zootechnical performance of shrimps.

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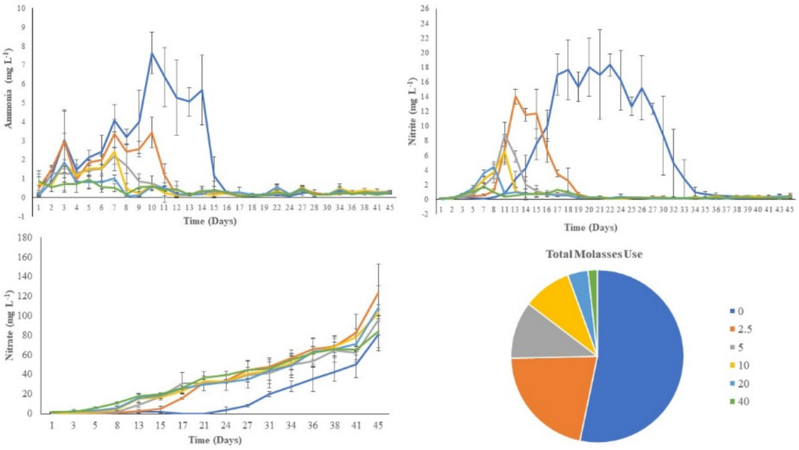

Figure 1. Ammonia, nitrite, nitrate and molasses used by the 45 days of experiment in the treatments: 0: Control, no addition of inoculum; 2.5: addition of 2.5 mg L^{-1}; addition of 5 mg L^{-1}; 10: addition of 10 mg L^{-1}; 20: addition of 20 mg L^{-1}; 40: addition of 40 mg L^{-1} of biofloc, inoculum in the culture starting.

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USE OF FISHMEAL ANALOG IN THE DIET OF *Litopenaeus vannamei* REARED IN CLEAR WATER AND BIOFLOC SYSTEM

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Introduction

One of the limiting factors for aquaculture development is the lack of fish meal for the composition of the feeds. Biofloc Technology Culture Systems (BFT) is characterized by zero water exchange and super-intensive culture of shrimp in enclosed raceway type (Krummenauer et al., 2014). This type of system is considered environmentally friendly and avoids nutrient rich waste from polluting coastal waters. In the last years several authors have been reporting that in BFT systems is possible to decrease the total amount of protein based on fish meal in the feeds. And besides, some of these researchers have been working to decrease the fish meal concentration in the feeds (Tacon & Forster, 2003; Tacon & Metian, 2008)”given”:”Albert G J”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Forster”,”given”:”Ian P”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””}],”id”:”ITEM-1”,”issued”:{“date-parts”:[[“2003”]]},”page”:”181-189”,”title”:”Aquafeeds and the environment : policy implications”,”type”:”article-journal”,”volume”:”226”},”uris”:[“http://www.mendeley.com/documents/?uuid=85e0efcf-2656-47c1-adbd-c0513ba70163”]},{“id”:”ITEM-2”,”itemData”:{“DOI”:”10.1016/j.aquaculture.2008.08.015”,”ISBN”:”0044-8486”,”ISSN”:”00448486”,”PMID”:”8800584”,”abstract”:”The finfish and crustacean aquaculture sector is still highly dependent upon marine capture fisheries for sourcing key dietary nutrient inputs, including fish meal and fish oil. This dependency is particularly strong within compound aquafeeds for farmed carnivorous finfish species and marine shrimp. Results are presented concerning the responses received from a global survey conducted between December 2006 and October 2007 concerning the use of fish meal and fish oil within compound aquafeeds using a questionnaire sent to over 800 feed manufacturers, farmers, researchers, fishery specialists, and other stakeholders in over 50 countries. On the basis of the responses received, it is estimated that in 2006 the aquaculture sector consumed 3724 thousand tonnes of fish meal (68.2% total global fish meal production in 2006. As a new strategy for shrimp production in BFT system, the present work aimed to analyze the effect of the use of a Fish Meal Analog (FMA) in shrimp feeds in substitution to Fish Meal, in the culture of *Litopenaeus vannamei* in BFT systems. The present study aims to evaluate the inclusion of different percentages (0%, 50% and 100%) of fish meal analog, formulated with by-products from the bovine and poultry industries in its composition in *L. vannamei* culture in clear water and in biofloc system.

Table 1. Means (± SD) of performance of *L. vannamei* fed with diferents levels of fishmeal analog in diets in clear water (CW) and biofloc (BFT) system

	CW0%	**CW50%**	**CW100%**	**BFT0%**	**BFT50%**	**BFT100%**
Survival (%)	65.5±6.7^{b}	62.2±3.8^{b}	76.6±1.6^{ab}	82.2±7.7^{ab}	80±14.8^{ab}	90.5±2.5^{a}
Final Weight (g)	9.2±2.0^{a}	8.8±1.9^{ab}	7.4±2.2^{c}	8.6±2.1^{ab}	7.9±2.5^{ab}	7.5±2.3^{bc}
Final Biomass (g)	362.2±23.8^{ab}	330.9±33.6^{b}	343.3±33.0^{b}	428.0±27.5^{a}	383.6±25.4^{ab}	412.3±19.8^{a}
Yield (kg m^{-3})	2.4±0.1 ^{ab}	2.2±0.0^{b}	2.2±0.2^{b}	2.8±0.1^{a}	2.5±0.1^{ab}	2.7±0.1^{a}
WWG (g)	1.1±0.4	1.1±0.4	0.9±0.2	1.1±0.4	1.0±0.4	1.0±0.4
FCR	1.2±0.0	1.2±0.0	1.1±0.0	0.9±0.0	1.1±0.1	0.9±0.0

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$). WWG:Weekly weight gain.FCR: Feed Convertion Rate.

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Material and Methods

The experiment was carried out at Marine Station of Aquaculture of Federal University of Rio Grande (FURG/ Rio Grande - Brazil). The study was conducted in eighteen experimental units with 0.15 m^3 each one, for 50 days. The system used for both treatments was the water recirculation, which consists in two matrix tanks with 7 m^3 of water each one, one containing clear water (CW) and the other biofloc system (BFT), where each matrix tank supplied water to others nine tanks, totalizing six treatments in triplicate: CW0% commercial feed in clear water; CW50% commercial feed with addition of 50% fish meal analog; CW100% commercial feed with addition of 100% fish meal analog, all of these in biofloc water (BFT). The density of storage was of 400 shrimps m^{-3}, with initial weight of 1.1 ± 0.5g. The statistical method of analysis of variance (ANOVA) was used to analyze water quality and production parameters (Zar, 2010).

Results

There were no significant differences in water quality parameters among treatments. Survival was highest in the BFT100% treatment and lowest in CW0% and CW50%. Final weight was highest in clear water (CW treatments), without statistically differing from CW50%, BFT0% and BFT50%. The highest final biomass was observed in treatment BFT0, without differing from treatments BFT50%, BFT100% and CW0%. The highest yield was observed in treatment BFT0%, without differing from treatments BFT50%, BFT100% and CW0%. FCR (Feed Convertion Rate) and WGG (weekly weight gain) didn't show significant difference among treatments (Table 1).

Discussion

All water quality parameters were within acceptable standards for *L. vannamei* (Crawford et al., 1999) cultivation and the inclusion of FMA did not change this standard.The higher survival in the BFT treatments, evidences the positive effect the biofloc system has on the cultured animals (Ray, 2012). Even using the fishmeal analog at a 100% level there was no significant difference in FCR among treatments, indicating acceptability of the feed containing fish meal analog to shrimp. The set of zootechnical performance parameters indicate that the substitution of fishmeal by the fishmeal analog may be more interesting in the biofloc system than in clear water.

Conclusion

It is possible to reduce the levels of fishmeal in the feed up to 100% in biofloc system without compromising the zootechnical parameters of the *L. vannamei*, as well as the water quality of the culture system.

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FISH SLUDGE – A PROBLEM OR A RESOURCE?

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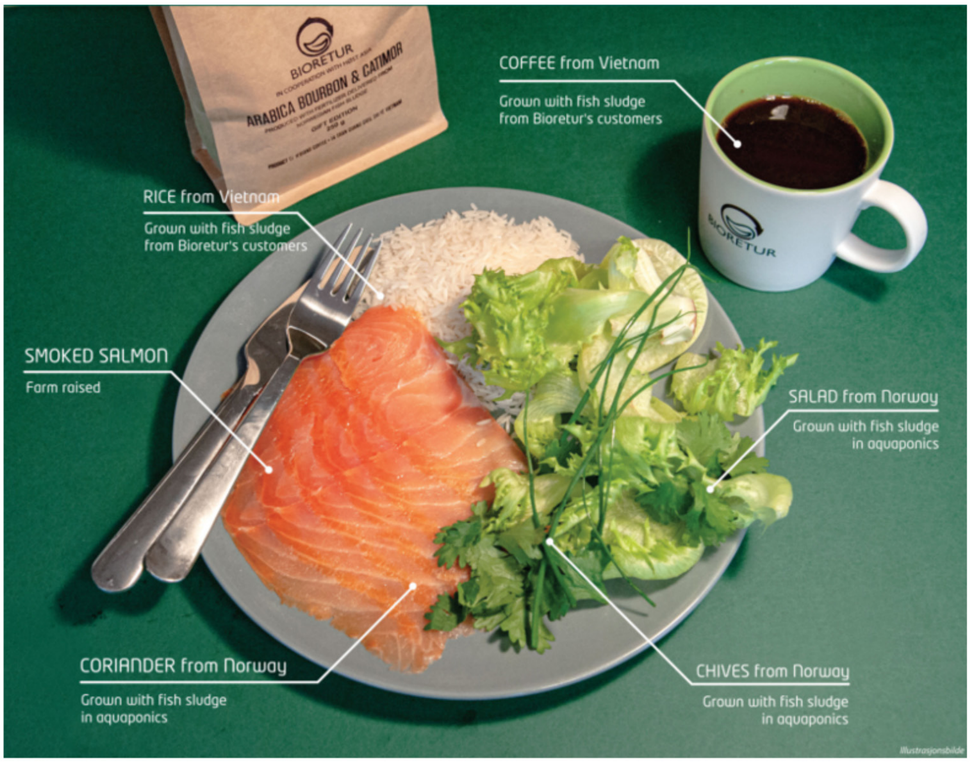
Fish sludge from land-based aquaculture facilities can be considered The Elephant in the Room, if this problem is not addressed and handled it can be a barrier for new land based project and further growth in marine food production. Fish sludge consists of feces and food waste from the production in the aquaculture facility, and is high on phosphates and other important minerals that is essential for sustaining life on the planet. Fish sludge also contains heavy metals such as zinc (Zn) and cadmium (Cd) and can thus not be used as is for fertilizer without special treatment and regulations. To be able to reach the climate goals, reuse of minerals such as phosphorus in a circular economy is essential, and we can simply not continue to waste thousands of tons of fish sludge every year.

Treatment of sludge in land-based aquaculture often consists of mechanical dewatering from 0,1 DS (dry solids) to around 15 % DS before it is transported to a biogas facility. Because the aquaculture sites often are far away from the biogas plants, this means transporting enormous amounts of water every year. This is highly expensive and not sustainable because it results in thousands of transport mileages. The use of fish sludge in production of biogas also results an high volumes of bio rest materials, with concentrated heavy metals.

The Norwegian company Bioretur offers a solution to the fish sluge problem and gives fish farmers access to the best available technology for sludge treatment for the aquaculture industry. At the same time they get a partner who ensures that the sludge is transformed from a problem to a sustainable resource. Bioretur are experts on sludge, and their value proposition is that for a fixed monthly fee they build, operate and maintain the technology of sludge treatment, while the client do what they do best: Produce high quality fish and seafood!

For the fist time, Steinar Wasmuth will present the Bioretur concept in EU.



PHYSICAL AND OPERATIONAL CONSTRAINTS MAPPING FOR EXPANSION OF OFFSHORE BIVALVE SHELLFISH AQUACULTURE

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Introduction

Increasing production and consumption of low-trophic filter-feeding shellfish could contribute to a reduction of food-related greenhouse gas emissions. However, on the supply side, there are many barriers to increasing low trophic marine aquaculture production, including the availability of suitable sites, competition for densely-used inshore waters, sufficient water quality, and regulatory hurdles. Expansion of aquaculture into more exposed coastal sites and more distant offshore waters represents an opportunity for a step change in production, particularly for low-trophic species (Krause *et al*. 2022). While global estimates of potential space available for offshore aquaculture development may be optimistic (e.g., 1,500,000 km^2 for bivalve shellfish production, Gentry *et al*. 2017), access to suitable offshore space will also be subject to a wide range of constraints. Offshore aquaculture development in European waters remains in its early stages, despite several pioneering R&D projects and successful commercial innovation at a range of sites. Both investment decisions and effective marine spatial planning for offshore expansion require an understanding of technical constraints in relation to environmental conditions. We have undertaken a characterisation of the hydrodynamics of UK and adjacent waters relevant for offshore mussel farms and mapped the sea space that is potentially suitable. Given the uncertainty regarding future technical innovation and how this may change operational constraints, we focused on the sub-surface suspended longline systems that are currently widely used in open water shellfish aquaculture.

Methods

Two approaches were applied to identify technical constraint criteria related to submerged mussel longline systems. Data were extracted from published literature identified in a systematic review, following CEE Guidelines for Systematic Reviews in Environmental Management and conducted using Web of Science Core Collection (1970-present). The identified set of key terms referring to offshore bivalve aquaculture were: 'offshore', 'suspended', 'longline' and 'open ocean'. Of 1,029 results, 101 were retained following the application of eligibility and exclusion criteria. Industry expert knowledge was also captured using a questionnaire approach. We surveyed shellfish producers globally to quantify key physical constraints to the installation and operation of commercial shellfish production in exposed and offshore conditions, using sub-surface suspended long-line systems. Based on the results of these approaches, quantification of physical hydrodynamic constraints (e.g., water depth, tidal currents, wave conditions) were combined with key operational constraints, to identify and map potentially suitable areas for shellfish culture around the UK coast.

Results

Review of literature data and operator expert knowledge yielded upper, and in some case lower, values for each environmental variable, giving a range within which existing farms are currently installed and successfully operated. The questionnaire also explored expert knowledge on upper limits that could constrain future expansion to more offshore/exposed sites. The results map a range of scenarios for the potential areas suitable for future expansion (see Figure 1 for an example scenario), testing their sensitivity to the various physical variables applied to the models and their interactions.

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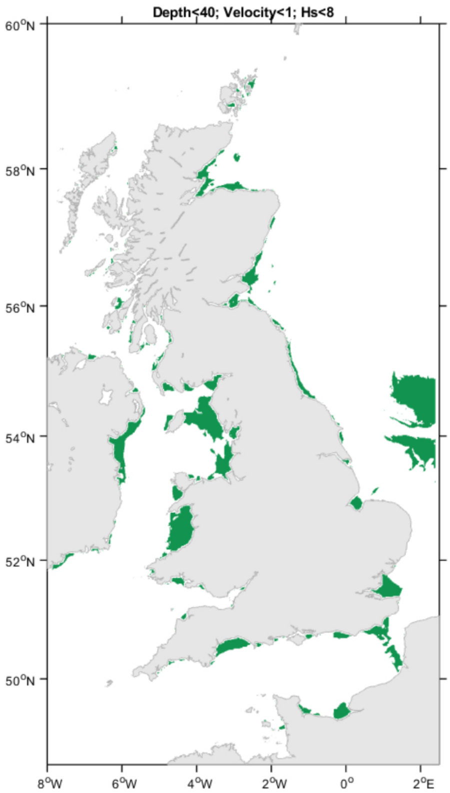

Figure 1. Example of suitable marine space available for offshore mussel longlines (green area) based on three physical constraints only; water depth <40 m LAT, tidal current velocity < 1ms^{-1} and annual peak significant wave height <8 m. In the poster, we present mapping outcomes based on a range of constraint limits and their interactions, showing the sensitivity of modelled outcomes in terms of available suitable space.

Acknowledgements

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HIGH QUALITY EARLY NUTRITION AS A STRATEGY FOR A PRODUCTIVE AQUACULTURE

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Early stage in life is a crucial step in fish development and impacts fish quality during their whole lifespan. As a consequence, this period must be managed very carefully. High quality nutrition during hatchery and nursery stage is part of the strategy to maximize fish performance. The ambition to improve aquaculture productivity up to harvest already starts in newly hatched fish.

In many species, it was demonstrated that nutrition at early stage impacts phenotypes during the entire lifespan. For example, piglets weaned too early (Dunshea et al., 2003) or trouts fed too fatty diets in the young phase (Alami-Durante et al., 2014) have their phenotypes modified at slaughtering with fatter body. In addition, key developments of organs happen in early life (Fujimura and Okada, 2007). That's why it is necessary to bring the right nutrients in sufficient amount during this phase. Muscle also starts to be built early in life and nutrition favoring a good muscle structure will promote larger body weight at harvest (Kiesslingetal et al., 2006). Even if the period remains short in comparison to an entire life cycle, the importance of a high quality nutrition should not be minimized as it is part of the strategy to prepare the future of the fish.

In order to demonstrate this statement, an experiment was conducted on tilapia during 236 days, starting from the first feeding. In this experiment, only the feed used during the first month of feeding was modified. One group was fed with a micro-extruded feed produced by marumerisation technology and the second group was fed a standard crumble feed during that period. The two groups were managed the same way and fed the same feed for the rest of the trial. A huge difference in live weight was observed after the first month since live weight was 3.17g and 0.52g ($P<0.0001$) in the groups fed the micro-extruded feed and the crumble feed respectively. The feed fed during the first month not only impacted performances during this period, but also performances up to harvest. Final live weight reached 797g and 683g ($P<0.0001$) after 236 days and fillet yield was 37.1% and 34.5% ($P<0.0001$) in the groups fed the micro-extruded feed and the crumble feed respectively. Survival was also strongly impacted. The benefit of micro-extruded feed using marumerisation technology on fish performances during the first month can be explained by a higher digestibility of the feed, a better stability in water and the possibility to feed the right pellet size adapted to fish mouth size while fish are growing. The benefit observed beyond is clear but needs to be further investigated. It might come from a better development of the fish at early stage leading to more resilient and more efficient animals. This experiment illustrates how the feeding management on the first month of life in tilapia can strongly affects fish performances during this first month and also how it impacts fish performances until harvest, 7 months later.

The importance of early feeding demonstrates why investment on high quality feeds at larvae and juvenile stages is part of the strategy to improve aquaculture productivity. With a positive effect on production efficiency, it is also supporting the reduction of the environmental footprint of the industry. Diversity in farmed aqua species requires early stage feeds to be specifically designed and formulated according to the requirements of each specie including shrimp, freshwater fish, marine fish and salmonid species to ensure precise nutrition.

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CONCERNS, CONFLICTS AND CONTROVERSY: UNDERSTANDING DRIVERS OF SOCIAL ACCEPTANCE OF SALMON AQUACULTURE IN NOVA SCOTIA, CANADA

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Introduction

Salmon aquaculture is a publicly controversial issue in Canada, with strong opposition being mobilized by certain groups such as environmental NGOs, fishing groups, and residents. Understanding social aspects are increasingly being recognized as important for more sustainable aquaculture management, fostering public trust and creating a positive environment for the growth of the industry (e.g., Krause et al., 2020). The present study was conducted to better understand public perceptions and opinions and identify factors influencing societal attitudes toward salmon farming, using Nova Scotia, Canada as a case study. This study investigated both public and community acceptance to help develop a framework of factors influencing social response to salmon farming.

Methods

This work is supported by two approaches. First, an online public survey aimed to capture the views and opinions of 495 Nova Scotians towards salmon farming. Describe statistics explored the influence of potential motivators on public opinion, including location, individual characteristics, personal value systems, exposure, trust, and perception of risks. Then, an ordinal regression model was fit to identify the most influential factor predicting more negative attitudes to aquaculture. The second approach conducted qualitative interviews with 24 participants across various interest groups within three salmon-farming communities in Nova Scotia to provide a comparative analysis of how locals have experienced aquaculture and identify common dimensions of conflict and controversy. Qualitative coding identified major themes in how locals perceive aquaculture. Major themes were compared across case study areas and across different perspectives on salmon farming.

Results

Survey results reflect a sample of the population largely in opposition, both towards salmon aquaculture as an industry and towards local development in the province. Rural and urban respondents were significantly different in their perceptions and opinions of aquaculture. Models identified five major drivers best predicting more negative views of aquaculture, including: age, environmental value system, salmon consumption patterns, trust in government, and perception of risks. Knowledge and exposure had little influence on opinion but may still help people who are undecided make decisions.

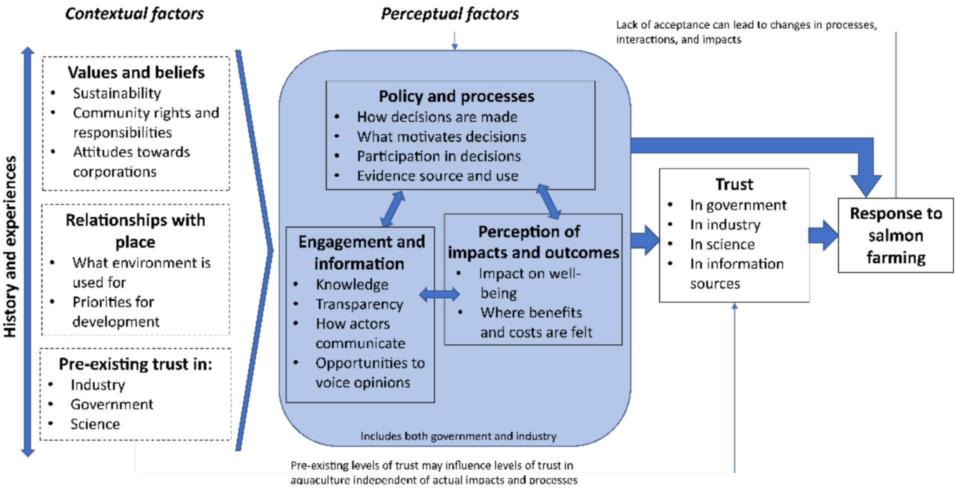

Fig 1. Framework of factors and feedbacks influencing public response (acceptance or opposition) to salmon farming.

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Findings from interviews with locals across three salmon-farming communities in Nova Scotia revealed a range of perspectives, experiences, and priorities toward salmon farming. Differences between case study areas revealed that perceptions about salmon farming reflected context-specific differences in history, relationships with place, and wider environmental and social values. Four distinct perspectives towards salmon farming emerged explaining the arguments and perceptions of participants. Contention emerged between the arguments made by supporters and opponents around the role salmon aquaculture plays including as a food system, as a tenant of the environment, as an economic provider, as a user of marine space, and how all are considered in the realm of aquaculture as a system to be governed. Perceptions across case study areas and different perspectives on salmon farming were synthesized to describe a conceptual framework of influencing factors on the social response to salmon farming (Fig 1). Across participants, the issue of trust emerged as a strong predictor of their response to salmon farming. Both trust and public response were dependent on perceptions across three core dimensions related to: 1) how decisions are made, 2) how stakeholders are engaged and informed, and 3) how benefits and costs from aquaculture are distributed.

Discussion

Drawing from this work, we find that social concerns about salmon aquaculture revolve not only around material (e.g., economic) well-being, but highlight the specific importance of relational well-being, being the relationships with family, community, and their environment. This work reveals how perceptions and attitudes are multifaceted, based on arguments around the multiple roles of aquaculture and how it is governed. Multiple perspectives highlight a complex and multi-faceted controversy around salmon farming recognized around the world (Young et al., 2019). Social acceptance of salmon aquaculture is highly tied to public trust and concerns over how the industry is managed. Still, findings reveal that opinion is nuanced across demographic and geographic contexts. Differences across rural and urban areas highlight the need to incorporate both perspectives in understanding conflicts and fostering acceptance. In summary, opposition does not represent a uniform set of experiences, concerns, or conflicts. Responding to opposition will require considering this variability and considering the underlying personal, social, and geographic contexts in which they are embedded.

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NOT A QUESTION OF HOW MUCH, BUT OF HOW AND WHERE: REDEFINING CARRYING CAPACITY FOR HOLISTIC ASSESSMENT OF AQUACULTURE

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Introduction

As food demands and priorities for aquaculture expansion increase around the world, conflicts around siting, planning, and management have become increasingly complex and require more holistic, integrated approaches to ensure sustainable environmental, social and economic outcomes. The Ecosystem Approach to Aquaculture (EAA) (Soto et al., 2008) outlines a management approach for aquaculture based on promoting equity and resilience of interlinked social and ecological systems. To do so, carrying capacity assessments have been proposed as a useful decision-support tool that could operationalize more interdisciplinary holistic assessments in support of an EAA (Ross et al., 2013). While traditionally carrying capacity for aquaculture is described to be how much aquaculture can be developed without unacceptable impacts on the environment, modern definitions also distinguish that it can be applied to study both physical, production, ecological, and social aspects. A better understanding of how carrying capacity could be developed for holistic assessment of aquaculture can help work toward more effective planning and decision-making in support of sustainable aquaculture governance and an EAA.

Approach

Here we present the results of a research program seeking to define a framework for a holistic assessment of salmon aquaculture. Broadly, the overarching goal of this research was to test whether carrying capacity can be operationalized towards more holistic management and planning. To do so, this work first conducted a comprehensive literature review on carrying capacity to explore the theoretical opportunities to mobilize the concept. Second, best-practice guidelines and recommendations were developed through Delphi consultation with expert practitioners and researchers from around the world. In light of research gaps on social aspects of carrying capacity related to social acceptance, this research also sought to understand the factors driving public and community perceptions of aquaculture, using Nova Scotia, Canada as a case study. Surveys with urban and rural residents across Nova Scotia investigated variables on public opinion to salmon farming, while interviews with residents and local stakeholders across three coastal communities in Nova Scotia explored how variables related to local context and experiences with salmon farming influenced perceptions. Findings across this research program were synthesized to develop a conceptual framework for holistic assessments of aquaculture limits.

Findings

Literature revealed that aligning carrying capacity concepts to more holistic management approaches require an approach that recognizes system complexity, responds to policy needs, applies adaptive frameworks, embraces interdisciplinarity, and curates meaningful information (Weitzman and Filgueira, 2019). In addition to the above, expert best-practices further emphasized the need for clear processes that include all relevant stakeholders (Weitzman et al., 2021). Expert consensus revealed a number of conceptual and knowledge barriers, especially around defining and understanding socio-economic limits and indicators. Results from public survey and interviews revealed differences in perceptions across demographic and geographic contexts. Perceptions also varied across contextual differences in history and experience with aquaculture, relationships with place, and environmental value orientation. The findings from this work revealed trust as a key indicator of social response to aquaculture. Expressions of trust and opinion were multifaceted but linked to arguments around perception of risks and benefits, interactions and knowledge of aquaculture, and procedures and processes.

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A way forward

Finding from this work highlight substantial theoretical and practical barriers to applying carrying capacity to more holistic, ecosystem-based management of aquaculture. This research argues that carrying capacity limits are inherently socially determined and thus a critical question of social carrying capacity. Recognizing that social aspects were a major roadblock for holistic approaches to carrying capacity, this research presents a definition of social carrying capacity for aquaculture. Social acceptance research argues that limits of aquaculture determined by society revolve not around a question of how much, but rather reflects a complex discourse around how the industry is managed and where benefits and risks are felt. Therefore, questions of aquaculture limits need to consider not only industry operations and impacts, but also the policies and procedures, and how society is involved and engaged. This, in combination with institutional and procedural barriers suggests that a comprehensive approach to carrying capacity assessment as a tool to further EAA may not be relevant, realistic, or appropriate.

Yet, this research presents a way forward to mobilize the intention and principles behind comprehensive carrying capacity by shifting towards a policy and decision-making lens that reflects more holistic consideration of 'Acceptable Limits of Aquaculture (ALA)'. Rather than replacing carrying capacity assessments, this lens aims to supplement it while adapting a more holistic vision for aquaculture management that accounts for an integrated view of ecological, social, and economic limits.

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HOLISTIC APPROACH TOWARDS SAFE AND SUSTAINABLE AQUACULTURE SUPPLY CHAIN

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OPTIMAL GUIDE TO PRODUCERS FOR GOOD AQUACULTURE PRACTICES AT ALL STAGES OF PRODUCTION

The tendencies on the global markets have been transforming and determining the demand for farmed seafood.

The GLOBALG.A.P. Aquaculture Standard version 6 brings to the market a complete solution for buyers and suppliers, based on current market demands, covering the key sustainability aspects that the animal production for human consumption is required to achieve. Operating since 2004, certified farms feedback reflects that this certification turns into a practical guide to their operations through detailed criteria written in a friendly and clear manner satisfying GLOBALG.A.P. purpose on working hand in hand with producers.

Although the ultimate goal is to obtain certification, the standard is the best tool the aquaculture sector can adopt to properly manage their operations. Currently there are 38 species certified for the finfish, crustaceans, and molluscs with coverage at all continents.

The aspects covered are those stipulated by the FAO Technical Guidelines on Aquaculture certification. With strong governance reliability, including the robust Integrity Program. This pioneering program is the first of its kind in food certification and is designed to ensure consistent delivery and implementation of the standard worldwide. It acts as a feedback mechanism that serves the ongoing improvement of the GLOBALG.A.P. System in all its aspects, promoting transparency and integrity.

The key elements of the GLOBALG.A.P. Aquaculture standard are currently recognized and evaluated.

- Food safety: is the ONLY certification scheme recognized by the Global Food Safety Initiative – GFSI for farming of fish.
- Environment: recognized by the Global Seafood Sustainability Initiative – GSSI Apr 2018.
- Animal Health: the OIE Aquatic Animal Health Code criteria for farms is covered by the standard. The robust Aquaculture Health Plan requirements is a reliable backing that farms give value.
- Animal Welfare: on top of animal health, GLOBALG.A.P. Aquaculture has been recognized as the only international private standard outside the UK that covers animal welfare practices at harvest and slaughter. Further animal welfare innovative criteria is spread for all production stages.
- Workers Occupational Health & Safety: workers are key to efficient operations, appropriate training is included in the requirements.
- Workers Welfare: GLOBALG.A.P. Risk Assessment on Social Practices is compulsory to assess.
- Compound feed used at certified farms shall be verified against the feed safety, responsible sourcing of feed materials, environmental and social criteria stipulated in the recently launched version 3.

The scope covers full production chain verification of: Broodstock, Seedlings, Feed, Farming Post harvest activities up to the point of sale for final consumers.

B2C communication: since the launch of the GGN Certified Aquaculture Label in 2016, the number of labelled products in the market have been doubling every year. The label can be found in many European countries as well as North America and Asia.

GLOBALG.A.P. As a member-based organization, counts with the support of retailer and food service members, supplier members and associate members. Retail members are committed to support safe and sustainable supply at primary production level.

The presentation intends to share to the audience the upgraded holistic approach for both GLOBALG.A.P. Aquaculture and Compound Feed Manufacturing standards.

IMMUNOMODULATATION OF FISH HEALTH BY BETA-GLUCANS

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Direct effects of β-glucans

Dietary supplementation of fish with β-glucans is commonly associated with immune modulation and is often considered beneficial for fish health. The exact mechanisms of immunomodulation by β-glucans in fish are stull under debate, however. The prototypical C-type lectin receptor (CLR) named Dectin-1 which directly senses the presence of β-glucans in mammals, appears missing from fish genomes. In our studies on common carp, direct immunomodulating effects of β-glucans on macrophages exist, at least *in vitro*, and could be the result of signalling mediated by one or more candidate β-glucan receptors, all members of the CLR family. Also, β-glucan-induced regulation of gene expression profiles typical of the CLR signalling pathway confirm C-type lectin receptors could indeed play a role in the direct sensing of β-glucans in fish.

Long-lived effects on innate immunity

Trained immunity is a form of innate immune memory which can possibly be induced by feeding β-glucans. Given the evolutionary position of fish as early vertebrates with a fully developed immune system, trained immunity should apply and include the ability of fish macrophages to mount heightened responses to a secondary stimulus in a non-specific manner. At least *in vitro*, carp macrophages can indeed be trained by β-glucans. Trained unstimulated macrophages display evidence of metabolic reprogramming as well as heightened phagocytosis and increased expression of the pro-inflammatory cytokines. Trained and re-stimulated macrophages showed heightened production of reactive oxygen and nitrogen species. Trained immunity could play a role in long term health-promoting effects of β-glucans in fish, but requires *in vivo* evidence, which is difficult to provide outside the laboratory.

Indirect effects of short chain fatty acids

Accumulating evidence supports a role for intestinal microbiota and their metabolites in the immunomodulatory effects of β-glucans. Yet, a proven relation in fish between types of diets including β-glucans, and immunomodulation *via* intestinal microbiota and associated metabolites requires more detailed investigations. Initial 16S rRNA sequencing of the active naive microbiota of carp intestine suggests the presence of bacteria with the capacity to degrade and ferment carbohydrates. This suggests that carp intestinal microbiota can ferment dietary β-glucans, a process leading to the production of short-chain fatty acids (SCFA). This important class of metabolites has been shown to affect the mammalian immune system in different ways. We could show that SCFA such as acetate, butyrate, and propionate, have immunomodulatory effects on carp leukocytes, including nitric oxide production and expression of several cytokines, at least *in vitro*.

Role of short chain fatty acid receptors

Not only *in vitro* data but also preliminary *in vivo* observations provide support to a role of SCFA in β-glucan-induced immunomodulation. A single oral gavage of carp with β-glucans modulated the local expression in the intestine of putative SCFA receptors. This could help explain immunomodulation by β-glucan *via* SCFA receptors present on intestinal leukocytes. In mammals, SCFAs can affect host cells via ligation to G-protein coupled receptors (GPR). Using genomic resources and cDNA cloning we identified several coding sequences for *gpr40*-like genes of carp. Phylogenetic analysis shows the carp *gpr40L* genes have a closer phylogenetic relationship to mammalian GPR43 than to mammalian GPR41.

Future developments

A first *in silico* screen of genomes of 25 different fish species largely confirms the observations for *gpr40L* of carp with regards to both, subdivision in three subclasses and conservation of synteny. Our data provide a further step towards an understanding of the function of SCFA receptors and their role in immunomodulation by β-glucans in fish.

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THE MOVE TOWARDS CIRCULARITY

Geert Wiegertjes, Yolanda Morales, Roy Koushik, Brian Thomsen

Wageningen University

The Circular Economy and its related Action Plan is high on the European political agenda and the subject of several research initiatives. But how does policy translate to practice and what does circularity really mean for aquaculture? Moderated by Geert Wiegertjes of Wageningen University, this panel discussion will address important questions that need answers, if this concept is to be enshrined in European aquaculture production practices. Panellists: Yolanda Morales, Roy Koushik and Brian Thomsen.

Geert Wiegertjes is professor of the Aquaculture and Fisheries group at Wageningen University, the Netherlands. As head of the research group, his scientific interest in the interaction between fish nutrition and health has broadened to include the multiple aspects of sustainable aquaculture. In his TEDx talk of 2020 he underscored the need for a sustainable production of fish and shellfish products in a world where demands for seafood keep rising. Geert Wiegertjes has been project coordinator and/or training coordinator of three MSCA training networks (FP5, FP7) and work package leader of Imaquanim (FP6), ParaFishControl (H2020) and currently, AQUA-FAANG (H2020). He coordinated TargetFish (FP7), one of the larger research consortia on fish health and vaccination. This year (2022), he announced the Wageningen University & Research (WUR)-wide ambition for WUR to be a major player on research and education in aquaculture and launched Wageningen Aquaculture Research & Education (W-ARE).

Yolanda Morales – ACUIPlus

Yolanda Morales is the manager of the ACUIPLUS cluster in Spain. ACUIPLUS is a group of companies and other stakeholders that promote the competitiveness and sustainability of the value chain of suppliers, including both products and services, and strengthening commercial, research, and innovation processes. and sustainability. ACUIPLUS supports its partners both nationally and internationally, promoting successful strategies, both individually and collectively.

Yolanda has a degree in Business Management and Administration and Master's Degree in International Studies. Her expertise includes revitalizing rural and coastal areas, formulation and implementation of projects in pursuit of rural productive development and the preparation of feasibility studies, market and sectoral strategic plans for economic growth. She is also an expert in design and implementation of CSR policies.

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Roy Koushik - University of South Bohemia

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CONCERNS AND RESEARCH PRIORITIES FOR THE WELFARE OF SCOTTISH FARMED ATLANTIC SALMON *Salmo salar* – AN INDUSTRY PERSPECTVE

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1. Introduction

Farmed salmon welfare is inextricably linked to the farming practices and conditions within the salmon farming industry (FAWC, 2014; Noble *et al.*, 2018). The intensification of Scottish salmon farming has been associated with increasing demands for the monitoring and safeguarding of farmed salmon welfare. Continued growth of farm productivity, while avoiding adverse effects on salmon welfare, will require the development of effective and time-efficient welfare assessment tools. Farming of Atlantic salmon involves rearing conditions, husbandry practices, responsibilities and welfare considerations that are specific to each production stage (Bergqvist and Gunnarsson, 2013; Noble *et al.*, 2018). When forming opinions on welfare concerns within this context, on-site experience of these various stages can provide important perspectives on current practices and the relevant challenges that are faced. This study involves a survey of the Scottish salmon farming industry, which was conducted to understand current salmon welfare concerns and priorities for research. The survey intentionally focused on industry stakeholders to provide insights into current practices and challenges associated with monitoring and assessing salmon welfare. This survey presents the first collection of opinions from professionals employed across the Scottish salmon farming industry regarding the current state of farmed salmon welfare, and where future research can best benefit salmon welfare and farm productivity.

2. Materials and methods

Multiple iterations of the questionnaire were first piloted through key informant interviews, both in-person and online. The final questionnaire, consisting of 53 questions divided into 6 separate question sections was carried out online. Background variables of participants were also recorded. A total of 61 individuals working in the Scottish salmon farming sector were recruited through the authors' industry contacts, online advertisements, and company mailing lists for the final survey. Participation was also voluntary. Various topics relating to the current state of Scottish farmed Atlantic salmon were investigated, including participants' largest welfare concerns, husbandry practices requiring the most attention for safeguarding salmon welfare, rating the suitability of current welfare measures for on-farm use, and rating the need for certain research priorities specific to on-farm welfare monitoring. For 3 of the question sections, participants were allowed to list and rank their own open-ended responses to prevent bias being introduced to the survey through limiting their responses. For question sections investigating more specific topics, questions were strictly quantitative (either through ratings or rankings) to allow for more in-depth statistical analyses to be carried out using IBM SPSS Statistics. Participants were also allowed to provide comments in addition to their ratings, which were used in a thematic analysis carried out in Nvivo. Where there was no clear consensus in responses to question sections across all participants, we assessed whether any difference in responses were significantly correlated with participants' professional backgrounds.

3. Results

The survey results highlighted the multitude of considerations that have to be made when monitoring and safeguarding salmon welfare. Participants have identified key areas of focus for salmon welfare specific to each major production stage and husbandry practice. Sea lice, gill health, and environmental challenges were of particular importance (Fig. 1 below). The results from this survey have also demonstrated the importance of using an integrated approach to welfare assessments that combines behavioural, physiological, and production-based parameters. Non-invasive, remote monitoring was identified as a priority for further development in welfare assessments, owing to their practical advantages and limited impacts on salmon welfare. Although behavioural measures were highlighted as one of the promising candidates for this role, participants noted that there are still many challenges (both practically and theoretically) that any animal-based welfare measures face. This study has helped highlight how newly developed welfare measures will only be adopted if balanced against the cost of their use.

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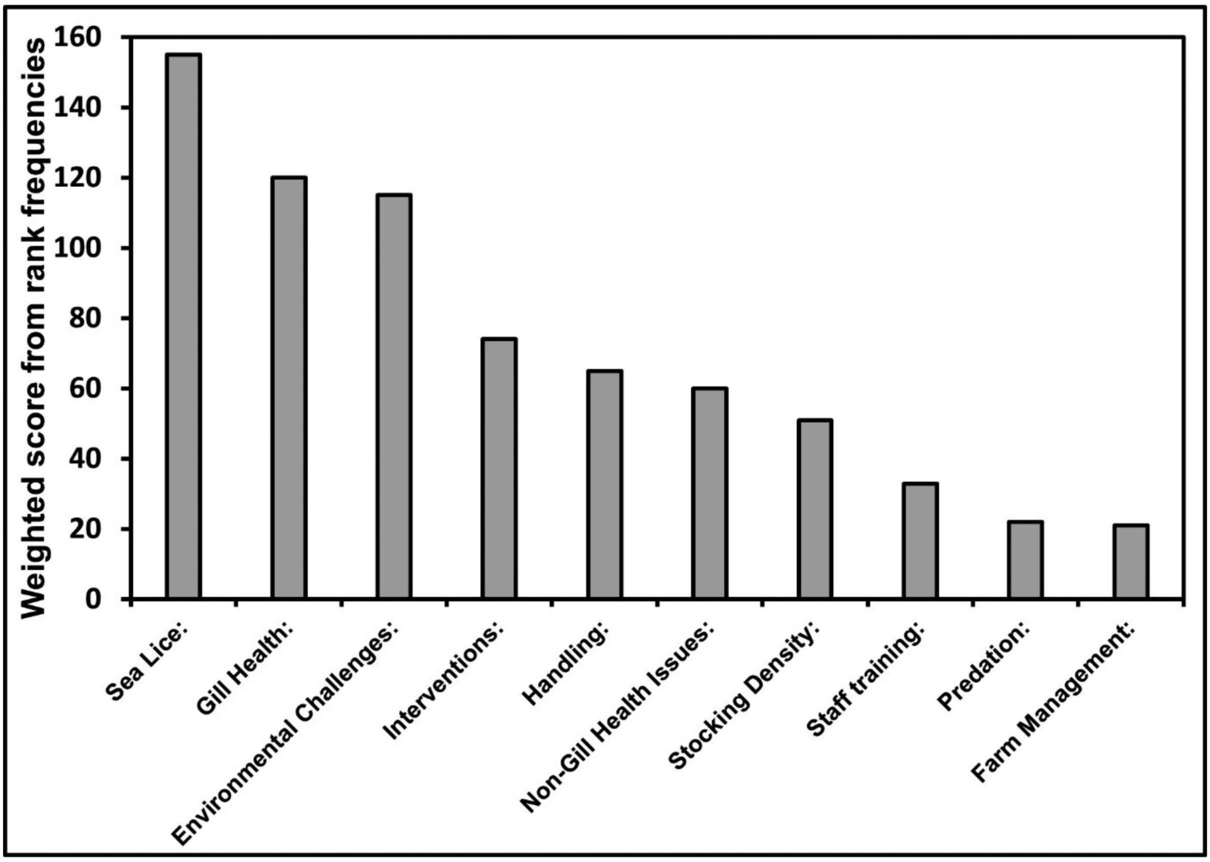

Figure 1: Top 10 ranked categories of overall farmed salmon welfare concerns (out of 16 categories listed), based on weighted scores provided by participants (n=61).

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OPPORTUNITIES AND CHALLENGES FOR UPSCALED GLOBAL BIVALVE SEAFOOD PRODUCTION

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Summary

Slow growth in the bivalve mariculture sector results from production inefficiencies, food safety concerns, limited availability of convenience products and low consumer demand. Here we assess whether bivalves could meet mass-market seafood demand across the bivalve value chain. We explore how bivalve production could become more efficient, strategies for increasing edible meat yield and how food safety could be improved through food processing technologies and new depuration innovations. Finally, we examine barriers to consumer uptake, such as food allergen prevalence and bivalve preparation challenges, high- lighting that appealing and convenient bivalve food products could provide consumers with nutritious and sustainable seafood options—and contribute positively to global food systems.

This abstract is based upon our recent publication in *Nature Food*, alongside unpublished data from research we have been carrying out with leading academics and global food companies. Please see Willer, D. F., Nicholls, R. J. & Aldridge, D. C. Opportunities and challenges for upscaled global bivalve seafood production. Nat Food 2, 935–943 (2021).

Benefits of unfed bivalve mariculture

To assess the sustainability and nutritional merits of bivalves relative to a current mass-market seafood, we compare bivalves with cod and Alaska pollock, alongside tuna (yellowfin and skip- jack). These wild-caught fish are already a proven resource and will continue to be a key component of consumer seafood, but supply is limited and global food companies are interested in how alternatives for filling the demand gap, such as bivalves, directly compare. In depth sustainability and nutritional analyses will be presented in the oral presentation alongside visual illustration.

Production

We discuss three key challenges to upscaling bivalve production: providing adequate volumes, maintaining sustainability, and ensuring food safety. We outline the emerging opportunities to tackle these challenges. These include new bivalve breeding approaches, feed technologies and grow-out practices; shell valorisation, the use of new species and sustainability regulations; and new depuration approaches including antimicrobial peptides, bacteriophages and chelating agents. We present several figures, including Figure 1, which outline the potential for production increases.

Food Processing

Bringing bivalve-based foods to the mass market will require creating bivalve products that can be easily and safely stored, distributed and purchased by consumers. We discuss the key challenges and opportunities in producing the major types of bivalve food products: fresh in-shell products, frozen de-shelled products, and cooked processed products. These are briefly outlined in Figure 2.

Consumer Uptake

We consider the key barriers to driving increased consumption of bivalves by the mass market and discuss the key opportunities to meet these challenges. We present the results of a consumer survey carried out with Europe's largest frozen food company (Nomad Foods) which can help us understand how to increase mass market demand. These results are compared with other global studies.

Conclusion

Demand for seafood is growing at double the rate of the global population, requiring nutritious seafood to be delivered to this mass market while avoiding damage to ecosystems through overfishing or poorly managed aquaculture. We discuss how through new innovation across production, food processing, and consumer uptake, bivalve shellfish can help meet this consumer demand.

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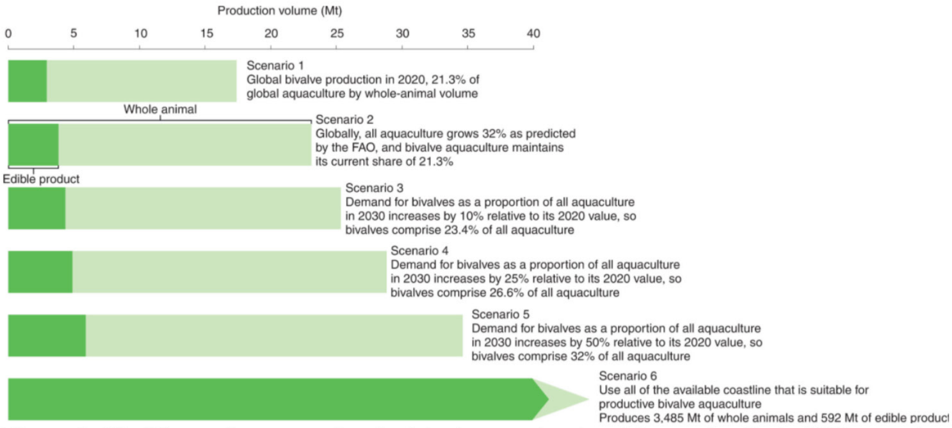
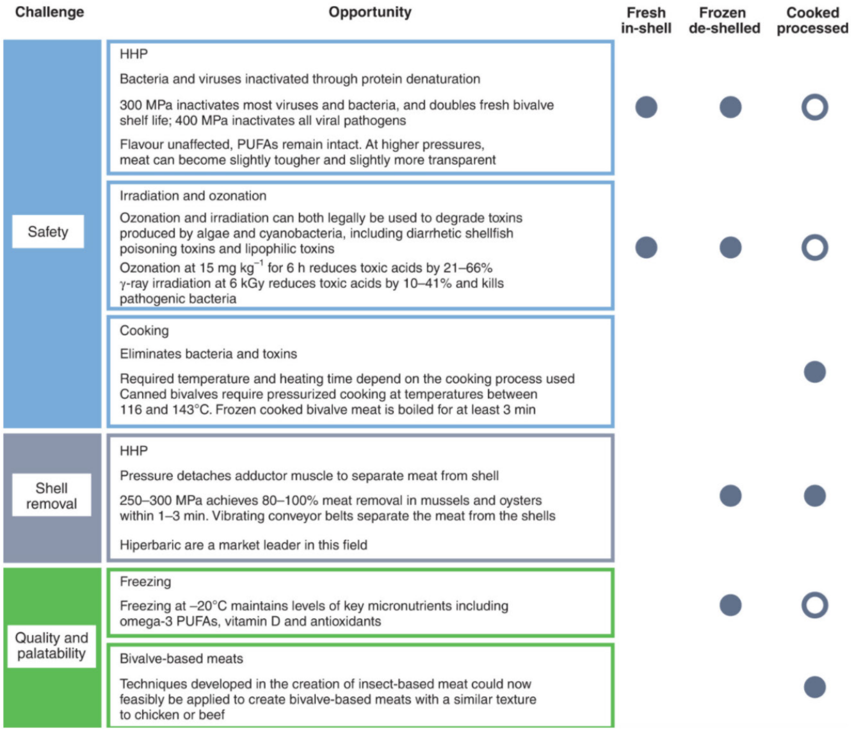


Figure 1. Six illustrative scenarios for bivalve production. Figure details in Willer et al. *Nature Food* 2021.



Challenge	Opportunity	Fresh in-shell	Frozen de-shelled	Cooked processed
Safety	HHP Bacteria and viruses inactivated through protein denaturation 300 MPa inactivates most viruses and bacteria, and doubles fresh bivalve shelf life; 400 MPa inactivates all viral pathogens Flavour unaffected, PUFAs remain intact. At higher pressures, meat can become slightly tougher and slightly more transparent	●	●	○
	Irradiation and ozonation Ozonation and irradiation can both legally be used to degrade toxins produced by algae and cyanobacteria, including diarrhetic shellfish poisoning toxins and lipophilic toxins Ozonation at 15 mg kg^{-1} for 6 h reduces toxic acids by 21–66% γ-ray irradiation at 6 kGy reduces toxic acids by 10–41% and kills pathogenic bacteria	●	●	○
	Cooking Eliminates bacteria and toxins Required temperature and heating time depend on the cooking process used Canned bivalves require pressurized cooking at temperatures between 116 and 143°C. Frozen cooked bivalve meat is boiled for at least 3 min			●
Shell removal	HHP Pressure detaches adductor muscle to separate meat from shell 250–300 MPa achieves 80–100% meat removal in mussels and oysters within 1–3 min. Vibrating conveyor belts separate the meat from the shells Hiperbaric are a market leader in this field		●	●
Quality and palatability	Freezing Freezing at –20°C maintains levels of key micronutrients including omega-3 PUFAs, vitamin D and antioxidants		●	○
	Bivalve-based meats Techniques developed in the creation of insect-based meat could now feasibly be applied to create bivalve-based meats with a similar texture to chicken or beef			●

Figure 2. Key opportunities for producing fresh in-shell, frozen de-shelled and cooked processed bivalve products. Figure details in Willer et al. *Nature Food* 2021.

LIFE CYCLE ASSESSMENT OF RAINBOW TROUT FARMING BASED ON THE TYPICAL FARM CONCEPT

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Introduction

Human food production contributes nearly 20% to global greenhouse gas emissions. Diet choices can significantly influence these greenhouse gas emissions. The environmental footprint of aquaculture largely depends on the production method and species cultured. In Europe, rainbow trout *Oncorhynchus* mykiss is one of the main species farmed while land-based flow-throug systems are the predominant production form. By using the streams natural hydraulic gradient such systems are considered to be relatively energy efficient compared to RAS.

In the recent study the ecological footprint of trout production in the temperate climate zone with respect to global warming potential, eutrophication potential, acidification potential, and ozone layer depletion was investigated. Data was collected under the typical farm framework and a cradle-to-gate life cycle assessment was conducted to find the processes contributing the most to the emissions of a typical trout farm. Two further scenarios were than analyzed, with either differing components in the fish feed or a differing amount of photovoltaic systems used on farm.

Material and methods

To estimate the environmental impact of 1 kg live weight (LW) rainbow trout in a traditional flow-through system four impact categories were analyzed: Global warming potential (GWP), Ozone layer depletion (ODP), Acidification (AP) and Eutrophication potential (EP). The impact categories were chosen due to their direct effect not only on the environment but also on human health. Data, which originated from a typical trout farm in South Germany, was considered with respect to energy and material flows. The model farm rears rainbow trout *Oncorhynchus mykiss* from egg to market size in three different river systems. ~500tonnes of trout are produced per year.

The chosen impact categories were analyzed with the software program SimaPro 9.1.1.1, using the CMLIA baseline/EU 25 method. Furthermore, the databases Ecoinvent v. 3.6, Agrifootprint v. 5.0, and Agribalyse v. 3.0 were used. To test for uncertainties, a Monte-Carlo simulation was run in SimaPro with 5000 runs to the 95th confidence interval

The model farm uses feed with 35% fishmeal and ~8% of the production site is covered with photovoltaic systems. The FCR of the fed feed is 0.89 (farmer's calculation)

For the analyses two further models were created. One scenario considering either no fishmeal (No FM) or 61.8% fishmeal (61.8% FM) in the feed, compared to the current model farm (35% FM). Different FCRs were considered for the differing formulations. The other scenario considering either no (No PV) or 100% (100% PV) coverage of the production site with solar panels compared to the current model farm (~8% PV). Here, different amounts of excess energy were considered as well.

Table 1: Results of the evaluated impact categories for the different scenarios (PV = Photovoltaic, FM = Fishmeal, GWP = Global warming potential, ODP = Ozone layer depletion, AP = Acidification potential, EP = Eutrophication potential)

Impact categories	GWP (kg CO_{2eq})	ODP (kg CFC_{11eq})	AP (kg SO_{2eq})	EP (kg PO_{4eq})
No PV	1.81	1.23e-7	0.0068	0.0282
No FM	1.63	7.89e-7	0.0061	0.0272
~8% PV/ 35% FM	1.18	7.89e-8	0.0055	0.0257
61.8% FM	0.84	6.19e-8	0.0054	0.0248
100% PV	0.77	5.92e-8	0.0046	0.0238

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Results

The production of 1 kg LW trout is most environmental friendly with a share of 61.8% fishmeal in the feed and a 100% coverage of the production site with solar panels (Table 1). The current model farm (35% FM, ~8% PV) emits around 1.18 kg CO_{2eq}. For the fish feed production itself, the no fishmeal variant emits 1.75 kg CO_{2eq}, while the 61.8% fishmeal variant emits 1.13 kg CO_{2eq}. Main shares of the emissions of feed production come from wheat, soy and fishmeal.

Discussion

The 1.18 kg CO_{2eq} of the recent study are in the lower range of values found in literature of farmed rainbow trout in flow-through system. Compared to terrestrial farming traditional trout production has a comparably low impact on climate. Nonetheless, there is room for improvement. Especially through the use of renewable energy sources and by improvement of feed formulations. Photovoltaic systems can offer the possibility to drastically reduce emissions by the production of excess energy. Additionally, the installation of roofs for PV-systems provides further benefits, such as temperature reduction by shading or the protection against predators. Regarding feed composition fishmeal seems to be the most environmental friendly solution, although this has to be considered carefully. The fish in: fish out ratio decreased within the last years, as fish meal and fish oil are more and more substituted by mainly plant based ingredients. Nevertheless, the increase in aquacultural production lead to a rise in fishmeal use, which can affect wild populations. According to the FAO, more than 50% of marine stocks are fully exploited. Vice versa, the use of some plants as fishmeal replacement can lead to high GHG emissions, mainly related to cultivation, deforestation and land use. Furthermore, some of the mainly used plants in fish feed need to by highly processed to be well digestible for fish.

In summary, it can be stated, that production of *Oncorhynchus mykiss* in Europe could be furthermore reduced by increasing the use of renewable energy sources such as photovoltaic and by reducing the impact of feed as the main single factor for most impact categories. Here, the use of modern technologies such as genetic manipulation of plants or sustainable insect meal could have the potential to decrease the environmental footprint of fish feed production.

ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*, L.) LARVAE ANTIOXIDANT MOLECULAR FUNCTIONS INDUCED BY DIETARY SELENIUM IN ROTIFER *Brachionus rotundiformis*

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Introduction

Recently the production cycle of Atlantic bluefin tuna (ABT) has been closed however with low survival at larval stages (Van Beijnen, 2017). Better knowledge of nutritional requirements could be one solution to boost ABT production. Selenium (Se) is an important micronutrient needed to produce selenoproteins involved in the antioxidant metabolism. The Se requirement in ABT remains unknown, but recent studies suggest that rotifers as starter feed do not provide sufficient Se for marine finfish species (Hamre et al., 2008). In general, fish exposed to high levels of stress have higher Se requirements (Küçükbay et al., 2009). Therefore, a supplementation at early stages, sensitive towards changing environmental conditions, might prove especially beneficial in ABT.

Material and Methods

The ABT eggs used in this study originate from ABT broodstock maintained in a floating net cage located at El Gorguel, off the Cartagena coast. From 2 days after hatching, larvae were fed with rotifer (*Brachionus rotundiformis*) grown on Algamac 3050 (Pacific Trading LTD, Kent, England) and enriched with different levels of Se (SelPlex®, Alltech, Meath, Ireland) for 18 h pre-harvest: 0.0 mg/10^6 rotifers (0 µg Se·L^{-1}, Se0), 3 mg/10^6 rotifers (3 µg Se·L^{-1}, Se3), 10 mg/10^6 rotifers (10 µg Se·L^{-1}, Se10), 30 mg/10^6 rotifers (30 µg Se·L^{-1}, Se30) and 100 mg/10^6 rotifers (100 µg Se·L^{-1}, Se100). The performance of ABT larvae was monitored throughout two weeks feeding period and antioxidant and selenoprotein expression was measured as well as Se contents on whole larvae sampled at the end of the trial.

Results

Rotifers accumulated up to 30.05 µg Se·g^{-1} dry weight in Se100, the highest supplementation level. Similarly, ABT larvae had maximum tissue Se concentration (194 ± 38 µg Se·g^{-1} dry mass) in Se100. In ABT larvae total length was highest in Se3, whereas maximum flexion index was reached in Se10. Selenium supplementation increased the expression of selenoproteins glutathione peroxidase 1 (*gpx1*) and methionine sulfoxide reductase 1 (*msrb1*) in Se supplemented treatments compared to the non-supplemented control (Figure 1). The expression of thioredoxin reductase 2 (*trxr2*) and selenoprotein P (*selenop*) also displayed differences between larvae fed supplemented and unsupplemented rotifers, albeit these were minor and varied with supplementation level (Figure 1). In contrast, the antioxidant enzymes catalase (*cat*) and superoxide dismutase (*sod*) showed lowest expression in Se100. The expression of other selenoproteins including iodothyronine deiodinases 1, 2 and 3 (*dio1*, *dio2*, *dio3*) was unaffected.

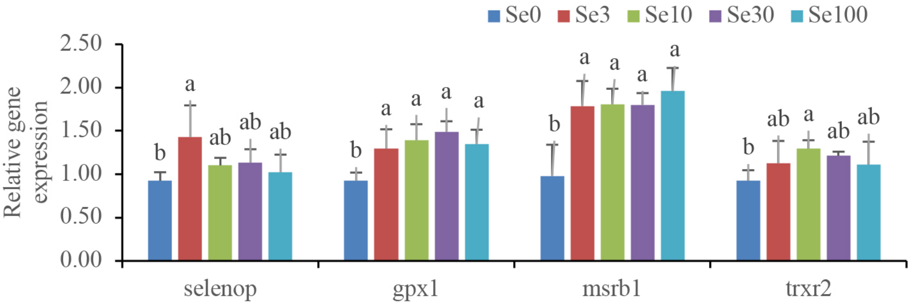

Fig 1. Expression of selenoproteins measured by real-time PCR. Data are normalized to a geometric mean of two housekeeping genes *ef1a* and *bactin* and expressed as fold-changes of mRNA abundance compared with Se0 (Pfaffl et al. 2002). Bars represent means ± SD (n=6). Means not sharing a common superscript letter are significantly different ($p < 0.05$) according to one-way ANOVA on ranks followed by Tukey's HSD. *Selenop*, selenoprotein P; *gpx*, glutathione peroxidase; *msrb*, methionine sulfoxide reductase; *trxr*, thioredoxin reductase.

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Discussion and conclusion

Feeding Se enriched rotifers effectively increased Se in ABT larvae tissue. The improved growth observed in Se supplemented treatments might be related to an accelerated development as the flexion index was significantly higher in all Se enriched treatments compared to the non-supplemented control. A similar effect by Se supplementation has been previously described in Senegalese sole (Solea senegalensis) in relation to an enhanced thyroid hormone activity by Se supplementation (Ribeiro et al., 2012). The Se level of 0.10 μg g^{-1} dw measured in non-supplemented rotifers is below known requirements in fish (Antony Jesu Prabhu et al. 2016). In contrast, rotifers supplemented with the lowest Se level (Se3) contained 4.42 μg Se g^{-1}, which might be sufficient to cover requirements for this mineral as selenoproteins displayed maximum expression in ABT larvae fed this treatment. The increased seleno-enzyme production might have contributed towards an improved antioxidant status in ABT larvae, indicated by a transcriptional downregulation of redox sensitive antioxidant enzymes *cat* and *sod*. In conclusion, rotifers without Se enrichment are suboptimal for ABT larvae at first feeding. A dietary Se level of 4.42 μg g^{-1} dw is recommended as it boosted growth performance and improved the antioxidant status in ABT larvae.

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ASSESSING THE EFFECTS OF EARLY UTILISATION OF VEGETABLE-BASED FEED IN THE MORPHOLOGY OF LIVER AND INTESTINE FROM CHALLENGED ATLANTIC SALMON

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Introduction

The proportion of plant-based ingredients being used to replace marine resources in Atlantic salmon feed is in constant growth. Hence, the lipid profile of the diets is changing, situation which can affect the performance and health of fish. The application of nutritional programming in freshwater stages of Atlantic salmon showed benefits in feed efficiency and nutrient retention when using plant ingredients in the stimulus diet compared to marine during first feeding. Moreover, physiological adaptations were described at gene expression level in the vegetable stimulated group after challenged. These changes could have improved the use of nutrients as well as enhance the fish tolerance to vegetable-based feed. However, it is important to determine whether these benefits can be extrapolated to seawater stage and if a "booster" is needed. The aim of the present study is to evaluate the impact of using vegetable-based feed in early stages of Atlantic salmon, on the morphology of liver and intestine of challenged individuals during seawater.

Materials and methods

A nutritional programming trial was performed with Atlantic salmon from six families characterised by high and low pigmentation genotypes. Fish were distributed into four groups and exposed to two stimulus diets; with either marine or vegetable ingredients, both delivered within the first three weeks of exogenous feeding. Fish were maintained with a commercial diet until seawater and challenged again with a vegetable-based feed which was delivered until the end of the trial when they reached harvest size. Samples of liver, to evaluate lipid vacuolization; and intestine for an assessment of circular muscle thickness, enterocyte height and width and number of goblet cells per area of surface, were taken before, during and once the challenge was finalised in seawater.

Results

Prior to the beginning of the challenge, the vegetable stimulated group showed statistically significant higher intracytoplasmic vacuolization in liver, despite been fed with a commercial marine diet during the intermediate phase. On regards of anterior intestine, marine stimulated group presented higher number of goblet cells per area compared to the vegetable group, however, the results were not statistically significant. Further results from intestine morphology and the following sampling point, will be presented.

FARMED LUMPFISH BROOD STOCK - WHAT TO EXPECT?

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Introduction

The Atlantic lumpfish (*Cyclopterus lumpus*) is deployed as cleaner fish to control the sea lice numbers in salmon farming in Norway, the UK, Ireland, the Faroe Islands and Canada (Treasurer, J. W. 2018). The commercial production cycle usually starts with the collection of eggs from wild caught lumpfish which are then incubated on land-based facilities, where larvae can hatch and will grow to juveniles, ready for transport to the salmon sea cages. The use of brood stock lumpfish of wild origin is not only seasonal but can also have an environmental impact and can be a risk to the production facilities by potential transmission of pathogens. A F1 brood stock which is reared in the hatchery can overcome those disadvantages. The objectives of a brood stock project at Lofotyngel AS (former Arctic Cleanerfish AS) in Lofoten, Norway was to determine whether the eggs and larvae from hatchery reared brood stock are of equivalent quality to those produced by wild sourced lumpfish.

Materials and Methods

In 2019, the brood stock for the main trial consisted of 10 females of wild caught (WC) lumpfish and 10 females of 18 months old hatchery reared (HR 1) lumpfish which were selected by their maturation status and were ready for egg collection on the same day. The weight of the females, the volume of hand stripped eggs, egg diameter, developmental stage of embryo, the fertilization rate (F-rate), the hatching rate (H-rate) of larvae, length of larvae at hatch, length of larvae every second week until 35 days post hatch (dph), weight of larvae every second week from 35 dph till 84 dph and the survival rate of larvae (S-rate) were recorded. In 2020 in a second part, the eggs from 10 females of 2.5 years old hatchery reared (HR 2) lumpfish from the same family as the HR 1 brood stock were collected and incubated. Same data as in the main trial was documented. Unfortunately, in this part no records during larvae rearing exist. All data were expressed as mean ± standard deviation (SD) and were analysed with one-way parametric ANOVA followed by Tukey's test. A p-value of < 0.05 was regarded as statistically significant.

Results

There were significant differences between the lumpfish brood stocks (Table 1). The females of the WC brood stocks were significant heavier ($p<0.001$) and produced a higher volume of eggs ($p<0.001$) compared to both HR brood stocks. Between the HR brood stocks, the females of the HR 2 where significant heavier ($p<0.001$) and produced more volume of eggs ($p<0.001$) than the HR 1 brood stock. The WC and HR 2 females showed significantly larger eggs ($p<0.05$) and significant larger newly hatched larvae ($p<0.001$) than the HR 1 females but were not different compared to each other.The F- and H-rate were not different between the brood stocks and were high in all (Table 1).

During the first feeding period (till 35 dph) larvae of the WC brood stock were significantly larger ($p<0.001$), (Table 1). In the on growing period (from 49 dph) larvae from the HR 1 brood stock were significantly larger ($p<0.001$). The S-rate of the larvae was high in both brood stocks at 98.7 % and was not significantly different (Table 1).

Table 1. Comparison of the HR (hatchery reared) and WC (wild caught) brood stock (n=90, except female weight and egg volume is n=20). Data is shown as mean ± standard deviation. Numbers sharing the same letter showing no significant difference (p<0.05).

	WC	HR 1	HR 2
Female Weight [kg]	4.10 ± 0.89 a	1.60 ± 0.45 b	3.4 ± 0.45 c
Egg volume [ml]	588 ± 99 a	250 ± 46 b	435 ± 180 c
Egg diameter [mm]	2.41 ± 0.07 a	2.26 ± 0.08 b	2.44 ± 0.07 a
F-rate [%]	99.9 ± 0.1 a	99.9 ± 0.1 a	99.9 ± 0.1 a
H-rate [%]	90.1 ± 6.4 a	90 ± 6 a	89.9 ± 6 a
Larvae at hatch [mm]	6.41± 0.2 a	6.17 ± 0.12 b	6.37 ± 0.13 a
Lumpfish at 84 dph [g]	1.15 ± 0.17 a	1.47 ± 0.2 b	n.d.
S-rate [%]	98.71 ± 0.05 a	98.71 ± 0.07 a	n.d.

(Continued on next page)

Discussion

The size difference of the females between the brood stocks explains the differences in the produced egg volume as with increasing size of the females, the egg production increases (Fuiman and Werner 2002). The age difference between the brood stocks could be the reason for this size difference. HR females were 18 months and 2.5 years old while wild spawning females are usually 5-8 years old (Davenport 1985). In addition to the age, the HR females were first time spawners. All this facts could also explain the smaller eggs of HR 1 females, as smaller lumpfish females and first time spawners laying smaller eggs than larger and multiple spawners (Fuiman and Werner, Kennedy 2018). Egg size and larvae size is directly related and therefore the HR 1 females produce smaller larvae at hatch (Kjørsvik et al. 1990).

On the other hand egg size has no direct implications for overall egg quality, larval survival and growth (Kjørsvik et al. 1990). Looking at the quality factors of the eggs studied here, fertilization and hatching rate, all brood stocks seemed to have the same quality. Earlier studies showed that hatchery reared lumpfish eggs seemed to have higher nutrient content then wild lumpfish eggs due to the more nutritious diet on farms compared to the natural diet of adult lumpfish (Hamre 2015). They concluded that high nutrient content of the eggs from farmed fish may determine juvenile performance. In this study the performance during start feeding, growth and survival of the larvae, were similar. However, after 49 dph larvae of the HR had a higher growth which could be the result of the nutrient content of the eggs.

Conclusion

Even tough HR brood stocks produced fewer volume of eggs per female than the WC brood stock, a huge advantage is that all eggs are pathogen free and can be incubated and utilized. In contrast a certain amount of eggs from WC brood stocks need to be discarded. The older HR 2 females could be interesting as they produced more volume than the HR 1 females, with similar sized larvae as the WC females. On the other hand, the smaller larvae at hatch did not seem to be a disadvantage as well as larvae were larger at the end of the trial. If the older brood stock has a similar superior growth but with larger larvae at start that could be of big advantage and should be studied further. In addition it would be interesting to see the performance of lumpfish in the salmon cages at the end.

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IMPACT OF DIETARY CURCUMIN SUPPLEMENTATION ON FISH OXIDATIVE STATUS

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Introduction

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. Previous research on dietary curcumin supplementation showed an improvement on growth and health status in Senegalese sole and gilthead seabream larvae (Xavier et al., 2021; 2022). This polyphenol, extract from the roots of *Curcuma longa*, presents high antioxidant properties due to its molecular structure that can interact directly with pro-oxidant molecules (either from the phenolic OH group or from the CH_2 group of the β-diketone moiety). Therefore, the aim of this study was to understand if the supplementation of curcumin could promote turbot postlarvae robustness and ultimately improve growth performance and survival during the hatchery period.

Material and Methods

A growth trial was conducted in 27 days after hatching (DAH) turbot that were fed one of the three experimental diets: a commercial-like diet used as control (CTRL) and the two diets supplemented with different doses of curcumin (CURLOW and CURHIGH). After 23 days feeding experimental diets, fish were sampled to assess growth performance (n = 21), and gut was dissected to analysed redox status biomarkers (n = 8).

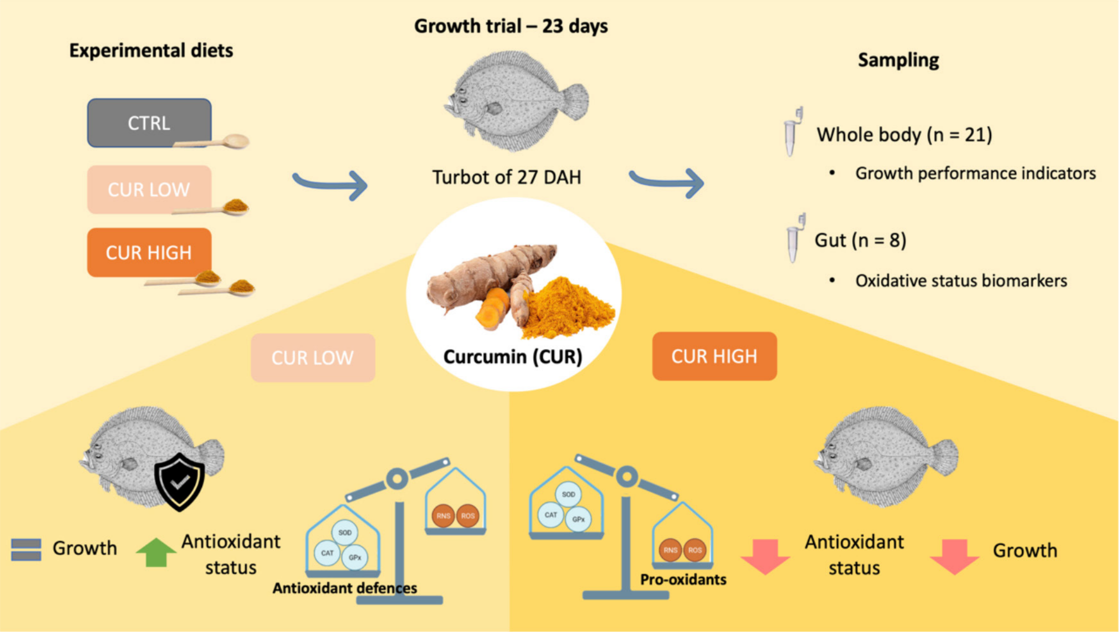

Figure 1. Graphical abstract of the present study.

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Results and Discussion

An overview of the experiential trial and main results are present in the figure 1. The higher dose of curcumin CURHIGH decrease turbot growth compared to fish from CTRL ($P < 0.05$). Concomitantly, CURHIGH postlarvae presented higher content of lipid peroxidation (LPO) and activity of catalase (CAT) than CTRL fish ($P < 0.05$). Which may suggest that a higher dose of curcumin is acting as pro-oxidant leading the fish to a state of oxidative stress. The lower curcumin supplementation was not able to promote fish growth performance when compared to CTRL fish. However, the CURLOW fish presented a higher total antioxidant capacity (TAC) when compared to fed CTRL fish ($P < 0.05$). A higher TAC may suggest that curcumin is acting as an exogenous antioxidant, and that in a challenging environment may help fish to cope better with adverse conditions.

Conclusion

The results from the study clearly demonstrate that curcumin presents the ability of acting as both antioxidant and pro-oxidant in turbot postlarvae. The inclusion of a moderate amount of curcumin seems to promote turbot postlarvae robustness through an increase in the antioxidant status. However, further evaluation is needed to identify the optimal inclusion level. Optimal curcumin dietary doses seem to change with fish species, and larval/postlarval age, when comparing the present turbot dataset with results for Senegalese sole (Xavier et al., 2021) and gilthead seabream (Xavier et al., 2022).

Acknowledgements

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AQUAPONICS PRODUCTION OF WHEATGRASS (*Triticum aestivum*) IN DIFFERENT HYDROPONIC SUBSTRATES WITH AFRICAN CATFISH (*Clarias gariepinus*)

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Introduction

Wheatgrass juice (a common addition to “smoothies” or as dried food supplement extracts) has become popular in recent years because it contains a variety of nutrients. Production of wheatgrass in aquaponics is rare. The aim of the present study was to test the influence of different aquaponic irrigation water from African catfish (*Clarias gariepinus*) production and various hydroponic substrates on the growth of wheatgrass (*Triticum aestivum*).

Material and methods

Wheatgrass was cultivated in an ebb-and-flood commercial aquaponic system from 30.10.2019 to 19.11.2019 in the FishGlassHouse of the University Rostock, Germany (Figure 1). Three different substrates were used, namely, 100% coconut fiber (C), 70% coconut fiber + 30% perlite (CP), and 50% perlite + 50% vermiculite (PV). Wheatgrass was irrigated by two different effluents from intensive aquaculture unit (IAU) and extensive aquaculture unit (EAU) of African catfish (*C. gariepinus*) recirculation aquaculture systems and tap water with fertilizer (Control) in triplicates. Plant growth indicators and some nutrients were taken and measured in order to evaluate the growth performance of wheatgrass produced in aquaponics.

Results and discussion

Data analysis showed that, regarding irrigation water, total fresh biomass and root dry mass had no significant difference among all groups. Shoot dry mass was significantly higher in fish water groups. Significantly different root length of all groups had the order of Control>EAU>IAU. The significantly highest SPAD-Index was discovered in IAU. Regarding substrates, the highest SPAD-index value was found in PV and not significantly different from CP. The significantly lowest mortality was in PV. Considering interaction of irrigation water and substrates on wheatgrass, the significantly highest value of shoot fresh mass was in PV and EAU combination. Significantly higher shoot length was in IAU and C, PV combination and EAU and CP, PV combination. Root fresh mass was significantly lower in IAU and C, PV combination. Minerals contents had variation due to co-effect of irrigation water and substrates and coconut and vermiculite substrates had positive influence on mineral content. So as vitamins, however, vitamins production reached partly their highest amount in the combination of fish water with different substrates. Abundant microbes including beneficial PGPMs existing in the aquaponic system and organic form of nitrogen in fish units are assumed to lead to higher contents of some nutrients in fish units (Glick, 2012; Palacios et al., 2014).

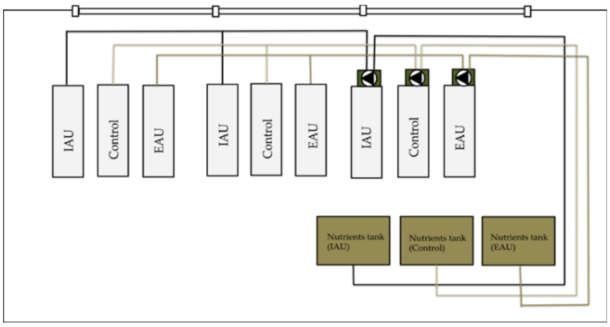

Fig. 1: Scheme of the hydroponics cabin. Bright grey rectangles represent plant tables; green rectangles represent sump tanks; and brown rectangles represent nutrient tanks; “◀”represents pumps.

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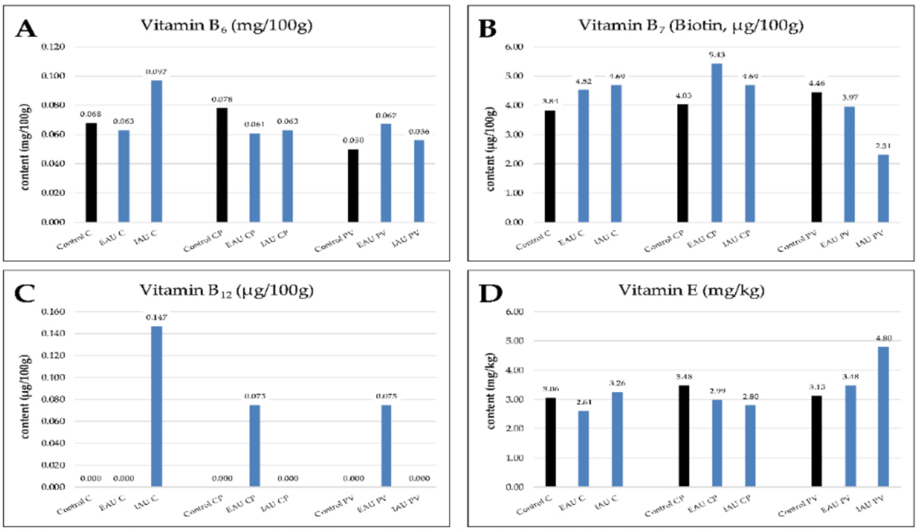

Fig. 2: Wheatgrass vitamin content of A) vitamin B_6, B) vitamin B_7 (Biotin), C) vitamin B_{12}, and D) vitamin E in different experimental groups (control, extensive aquaculture unit (EAU), and intensive aquaculture unit (IAU)); substrates are illustrated as coconut 100% (C), coconut 70% + perlite 30% (CP), and perlite 50% + vermiculite 50% (PV).

This finding showed the potential that aquaponic is a more sustainable way of producing vitamins under aquaponic gardening conditions in future (Palm et al., 2018). Studies of verifying the findings and examining the bacterial populations that inhabit the aquaculture effluents and substrates should be undertaken.

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PIKEPERCH (*Sander lucioperca*) LARVAL REARING OPTIMIZATION: UTILIZATION OF LACTIC ACID BACTERIA FOR IMPROVING MICROBIOME DIVERSITY AND DIGESTIVE ENZIME ACTIVITY

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Introduction

Probiotics are "live microbial feed supplements which beneficially affects the host animal by improving its intestinal microbial balance"(Fuller, 1989). Exposing fish larvae to selected probiotics has been proven to improve their health and increase their resilience to pathogens and disease due to the gastrointestinal microbiota dependency on the external environment (Gatesoupe, 1999). Probiotics also compete with pathogens for nutrients and adhesion sites, which help to stimulate the immune system (Gatesoupe, 1999).

Materials and Methods

This trial tested the use of lactic acid bacteria (LAB) on pikeperch (*Sander lucioperca*) larvae during first feeding. The trial included the use of two probiotic treatments and one control (no probiotics). The first treatment exposed the live feed to LAB, while on the second treatment, LAB was used on the live feed as well as in larval tanks. Pancreatic (α-amylase, bile salt-activated lipase, total alkaline proteases) and gastric (pepsin) enzyme activities were determined as well as the microbiome from the larvae and the rearing water during. The trial duration was 18 days and samples of larvae and water were taken at 7, 14, 21 dph from each treatment.

Results

Significant differences were found between treatments in terms of enzyme activity at 7days post hatching (dph) when looking at alkaline protease, amylase, lipase and pepsin. Further significance was found on lipase activity at 21 dph. The use of LAB also had significant differences on the microbiome abundance and diversity on the larvae, as well as on the rearing water. Results showed a significant variation of the microbiome with time, as well as a between treatments. Such effects might be the one of the main factors for the difference on survival between treatments. Larvae exposed to LAB via the live-feed and the rearing water had a significant higher overall survival rate (88%) than the other two treatments at 21 dph. At the same time, larvae exposed to the probiotic treatment had a significantly higher TL (7.34 and 11.82 mm) after 14- and 21-days post hatch (dph) compared to the control treatment (5.99 and 10.67 mm).

Conclusions

The results suggest that the use of LAB in both live feed and rearing water during pikeperch larval stage has a positive effect by enhancing early enzyme activity, controlling microbiome diversity and improving the growth and survival rates.

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USE OF CHLORELLA DURING PIKEPERCH (*SANDER LUCIOPERCA*) LARVAL CULTURE

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Introduction

Supply of live feed during fish larval rearing is of key importance to develop intensive aquaculture protocols. Recent work done on pikeperch *(Sander lucioperca*) larvae rearing, have revealed the need to introduce rotifers to their diet during the first feeding stage. The small size of this live feed is the main characteristic that makes them a suitable prey for pikeperch larvae, as opposed to the traditional artemia nauplii. Yet, rotifers nutritional value by itself lacks essential components, such as essential fatty acids. The diet given to rotifers is the main source of essential nutrients required by the larvae to develop.

Material and Methods

This trial compared the effects on pikeperch larvae survival and development from three rotifers diets, (*Nannochloropsis oculata, Chlorella vulgaris*, and a commercial enrichment diet). Three treatments were tested. The first was the control treatment, where larvae were offered rotifers (*Brachionus plicatilis*) fed with *Nannochloropsis occulata* during the first eleven days (15 dph), and unenriched artemia until the end of the trial. The second treatments followed the same feeding protocol as the control treatment, but rotifers (*Brachionus plicatilis*) supplied to the larvae were fed with *Chlorella vulgaris*. *Artemia salina* provided to larvae from the second treatment was not enriched, to assess the potential long-term effects of the *Chlorella vulgaris* supplementation on the larvae fatty acids' composition and survival. The third treatment used rotifers (fed on *Nannoclopropsis*) and artemia, both enriched with Spresso emulsion by Selco (INVE, Salt Lake City, UT, USA).

Results

Larvae fed on *Chlorella* had the highest survival and faster growth. Such result might be due to the highest (10 times higher) concentration of linoleic acid (LA) found in chlorella as opposed to *Nannochlopsis* and the ability that pikeperch larvae have to synthetized LA into docohexanoic acid. The use of chlorella for rotifer feeding could potential be a good candidate to replace other diets, lowering the production cost and increasing pikeperch larval production.

WHAT HAPPENED OVER THE LAST 10 YEARS (2012 to 2021): A BIBLIOMETRIC ANALYSIS OF FISH WELFARE RESEARCH IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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This paper describes fish welfare publications on European sea bass, *Dicentrarchus labrax* which is the most produced fish species in the Mediterranean region. To perform the bibliometric analyses, we downloaded the publications from the Web of Science (WoS) database using the keyword combination ‘‘fish welfare and European sea bass” for the period from 2012 to 2022 years. A total of 104 publications were extracted, including 12 reviews and 92 articles. Of the publications, almost all were SCI-expanded (n=102 publications). The visualization analysis in WoS showed that the highest number published in 2021 (n=22) and the lowest in 2014 (n=6). While the University of Algarve (Portugal) is at the top of production in the publications on fish welfare research in sea bass Spain has the highest number of publications. European Commission is the main funding source with a ratio of 32%. The results of this study clearly indicate that although the research on fish welfare of sea bass showed an increasing trend in recent years total number of publications produced the last decade was low. Considering that the importance of fish welfare from the perspective of consumers' perception, production feasibility, and aquaculture sustainability, is high, it is clear that the welfare studies on European sea bass should be increased in the research area of aquaculture.

GROWTH PERFORMANCE OF MEDITERRANEAN FINFISH SPECIES UNDER HIGH TEMPERATURES

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Introduction

Although Mediterranean Sea finfish species are becoming important in Saudi Arabia's aquaculture production because of diversifying aquaculture, the farming sector requires a sufficient understanding of the species' thermal preferences and limits. Moreover, High water temperatures have limited the culture species and periods in Saudi Arabia (Figure 1). This study was conducted using three finfish species juveniles (135–155 g), meager (*Argyrosomus regius*), European seabass (*Dicentrarchus labrax*), and greater amberjack (*Seriola dumerili*), to assess their growth performance under high temperatures for three-month-long thermal trials.

Methods

This study was conducted using three finfish species juveniles (135–155 g), meager, European seabass, and greater amberjack, to assess their growth performance under high temperatures for three-month-long thermal trials. Each species trial was performed in triplicates in a recirculating aquaculture system, while three temperature ranges, 24°C–25°C, 28°C–29°C, and 33°C–34°C, were tested, representing the average temperatures in the Mediterranean and Red Seas.

Results

Both meager and European seabass performed similarly between the first two temperatures, indicating that the optimum range lies within that thermal window. Growth performance indicates that it is lower for meager than it is for European seabass. In the greater amberjack, growth performance was similar for the first two temperatures. The temperature of tolerance was 33°C for all species.

Discussion

Consequently, the species appeared to have similar thermal tolerance to the upper end of the tolerance range but with some notable differences in the preferable temperature for optimum performance. This became evident from the husbandry findings and was further corroborated by the growth performance analysis. Both meager and European seabass performed similarly between the 24°C and 29°C, indicating that the optimum range lies within that thermal window. However, growth and FCR indicate that it is lower for meager than it is for the European seabass. In the greater amberjack, growth performance was similar for the first two temperatures. Yet, the high growth rate at 29°C during the second and third month of the trial suggest a high capacity for compensatory growth and the potential to achieve appreciable growth in high-temperature regimes, unlike the other species.

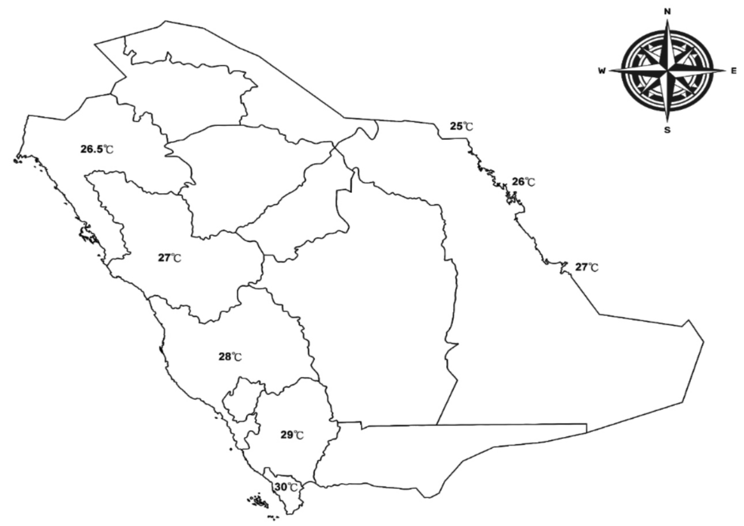

Fig 1 The average water temperature of location of primary aquaculture companies in Saudi Arabia

FARMED ATLANTIC COD: SEAWATER TEMPERATURE AFFECTS BIOLOGICAL PERFORMANCE, DISEASE DEVELOPMENT AND SITE SELECTION

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Introduction

Atlantic cod (*Gadus morhua* L.) is one of the most important species for commercial fisheries in the North Atlantic. However, cod catches and spawning stock biomass in the North Atlantic Ocean has declined the last decades, and traditional fisheries may become further reduced due to climate change (IPCC, 2022). This reduced availability of wild cod in the near future has renewed the interest in cod farming (Puvanendran et al., 2022). Cod farming used to be a thriving industry in Norway until it gradually declined from 2008 due to multiple factors. However, selective breeding and new knowledge about production have reinitiated cod farming in recent years. Novel methods and strategies that sensitively measure and predict cod health and welfare, as well as insight into how climate change will affect production, is vital for the sustainable growth of the industry.

Optimal temperature for mature cod is between 6 and 11°C. With temperatures above 15°C, several challenges related to slower growth, impaired swimming, increased stress and reduced haemoglobin oxygen affinity are described, with increased mortality when temperatures reach 20°C. The biological significance of these thermal thresholds is worth a closer look to understand the limitations, opportunities, and possible strategies of adaptation when planning to increase cod farming under climate change (Falconer et al., 2022). For example, water temperatures above 15°C seems to play a significant role in development of francisellosis. Francisellosis, caused by the intracellular bacterium *Francisella noatunensis*, is considered one of the most important disease problems in Norwegian cod farming and was one of the reasons why the industry collapsed around 2010. Current expectations of increased production could cause the disease to re-emerge as a challenge.

Using climate projections from the Intergovernmental Panel on Climate Change (IPCC) we have evaluated potential future temperatures at selected cod farms in Norway. Based on these results we designed a temperature study with Atlantic cod to evaluate biological impact from temperature alone and in combination with a *F. noatunensis* infection.

Methods

The climate projections from IPCC showed that long-term temperatures around 17°C are likely at selected cod farms in the near future. Trial with Atlantic cod was therefore performed at to two different temperature regimes, 12°C and 17°C for 8 weeks. Three weeks into the trial, a *F. noatunensis* infection was carried out.

Samples of skin and spleen were collected at different time-points, and microarray, histology, scanning electron microscopy and immunohistochemistry used to evaluate the impact of temperature on general health, and to determine the pathogenesis of *F. noatunensis* at different temperatures. An *in vitro* model of skin biopsies and scale explants was used to further investigate the functional biology of increased temperature. Migration assays and immunohistochemistry with markers for stress induced genes were used in combination with temperature and oxidative stress exposure of the cells to increase our understanding of combined stressors.

Results and Discussion

Results showed that temperature has a strong effect on skin barrier quality, affecting important functions such as reducing the immune status, proliferation rate and migration capacity, and making the skin less tolerant to oxidative stress. These are vital biological responses in fish skin, as they contribute to keep the fish healthy and robust in the long term. The effects caused by temperature were stronger than those caused by the *F. noatunensis* infection, but the infection had a more severe pathology in fish from the high temperature regime. Fish from the 17°C group showed more severe granuloma formation and developed granulomas at earlier time-points in the spleen, compared to infected fish from the 12°C group. These findings were further combined with the climate model projections to identify risks to existing cod farming areas, and implications for short and long-term cod aquaculture site selection.

(Continued on next page)

Conclusion

Results show that climate change will pose a significant threat to Norwegian cod farming, that important biological functions and responses in cod are severely affected by high temperatures, and that action is needed to sustain production in the future.

Acknowledgements

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PRELIMINARY EVALUATION OF BY-PRODUCTS FROM CEREAL PROCESSING AS ALTERNATIVE INGREDIENTS IN AQUAFEEDS FOR GREATER AMBERJACK (*Seriola dumerili*)

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Introduction

Plant-derived flours are currently being used as alternative ingredients to fishmeal in aquafeeds. However, most of them are competing for other uses, mainly as food for humans. The cereal industry generates abundant amount of residual by-products that may represent a source of sustainable and low-cost feedstock for fish feeding, presenting limited competition with human food consumption. In this study, four different by-products derived from the cereal industrial processing has been selected and tested to assess their usefulness as potential alternative ingredients in aquafeeds. With this aim, a generalist formulation was designed in order to check those ingredients in three different European fish species (Atlantic salmon, gilthead seabream and greater amberjack). Therefore, the feed formulation was not customized for any of the targeted species. The present report shows the experiments carried out with greater amberjack (*Seriola dumerili*).

Materials and methods

Greater amberjack juveniles (n=141; initial weight 60.50 ± 10.48 g) were randomly distributed in 9 tanks in recirculating aquaculture systems and maintained during 18 days at 22 ºC. Fish were fed with 5 isoproteic experimental diets at a daily feeding ration of 3.5% w/w, distributed in three meals. The experimental diets, manufactured by SPAROS Lda (Portugal), included 7-15% of by-products from cereal industry produced by ROQUETTE (corn germ, corn gluten feed, corn germ meal, and wheat gluten feed) and soy protein concentrate (control). The protein content of these alternative ingredients ranged between 15 and 23%. These ingredients replaced standard cereal flours and krill and squid meals. All diets were marked with yttrium oxide. Growth performance and apparent digestibility were measured at the end of the experiment. Previously, a short experiment was performed with feeding up to satiation to assess the acceptability of the diets. In addition, *in vitro* digestibility of novel ingredients and diets was measured after incubation for 1 and 6 h with intestinal extract of greater amberjack. Apparent digestibility was measured analyzing yttrium in the faeces collected from the *in vivo* experiment using inductivity-coupled plasma mass spectroscopy. Statistical differences were checked by one-way ANOVA followed by Tukey's test using RStudio software.

Results

All experimental diets were well-accepted and the fish grew up in all cases. Voluntary feed intake was similar for all diets, ranging from 4.71 to 5.88 % body weight day^{-1}. Likewise, no statistical differences were detected in final body weight (ranging from 98.5 to 114.7 g) among the experimental diets, including the control with soy protein concentrate. Thus, the weight gain during the 18-day experiment ranged between 63 and 90% of initial body weight.

The *in vitro* protein digestion of the experimental ingredients showed significant differences after 1 and 6 h of incubation ($p < 0{,}05$). However, when the experimental formulated feeds were considered, the differences were significant only after 1 h of incubation and not after 6 h ($p > 0{,}05$) (Table 1).

Discussion

All experimental diets were accepted and were able to support fish growth. Both, the *in vitro* and *in vivo* digestibility analyses of the diets indicated that *a priori* the protein of all the four new ingredients can be digested at similar efficiency than the control by greater amberjack. The problem may be the relative low protein content of these ingredients. A dose-dependent long-term growth experiment with a customized formulation for this fish species with some selected ingredients could confirm the usefulness of these by-products as more sustainable alternative ingredients for fish feeds. In first instance and according to the digestibility results with greater amberjack, wheat gluten feed (Milurex, produced by Roquette Beinheim, France) and corn gluten feed (Corex, produced by Roquette Benifayo, Spain) seems to be the most appropriate as alternative ingredients. Results with the same diets, fed to other species, Atlantic salmon and gilthead seabream, will be reported when available.

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Table 1.- Protein *in vitro* digestibility (µmol of amino acids released per mg ingredient or diet) of the novel ingredients and of the diets prepared with these ingredients after 1 and 6 hours of incubation with intestinal extracts from greater amberjack juveniles.

	Ingredients protein digestibility		**Experimental feeds protein digestibility**	
Ingredient	1 h	6 h	1 h	6 h
Corn germ	0.015 ± 0.008^{b}	0.025 ± 0.005^{c}	0.027 ± 0.004^{b}	0.070 ± 0.002
Corn gluten feed	0.016 ± 0.000^{b}	0.018 ± 0.003^{d}	0.030 ± 0.002^{b}	0.070 ± 0.009
Corn germ meal	0.021 ± 0.000^{b}	0.033 ± 0.003^{c}	0.033 ± 0.002^{ab}	0.072 ± 0.001
Wheat gluten feed	0.019 ± 0.001^{b}	0.049 ± 0.003^{b}	0.033 ± 0.004^{ab}	0.073 ± 0.010
Soy protein conc.	0.114 ± 0.001^{a}	0.186 ± 0.005^{a}	0.041 ± 0.004^{a}	0.083 ± 0.004

Data are presented as mean ± SD. Different superscript letters indicate significant differences among diets for each sampling point (One-way ANOVA; $p < 0.05$).

Table 2.- Apparent digestibility of the different diets on weigh basis (ADC) and digestibility of dietary protein (AD protein) by greater amberjack juveniles.

Diet with:	**ADC (%)**	**AD protein (%)**
Corn germ	77.60 ± 1.18	95.68 ± 0.32
Corn gluten feed	78.85 ± 3.11	95.88 ± 0.36
Corn germ meal	77.32 ± 2.59	95.16 ± 0.33
Wheat gluten feed	78.56 ± 2.35	95.41 ± 0.47
Soy protein conc.	76.37 ± 3.80	95.43 ± 0.71

Data are presented as mean ± SD. No significant differences among diets were found (One-way ANOVA; $p > 0.05$).

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EFFECTS OF TWO DIETS WITH DIFFERENT PHYTOESTROGEN CONTENT ON FILLET AND LIVER FATTY ACID COMPOSITION OF SEA BREAM (*Sparus aurata*)

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Introduction

Sea bream (*Sparus aurata*) is one of the most commercially important aquaculture species. The increased use of plant raw materials in the diets contributes to the increase of their phytoestrogen content. Major phytoestrogens found in fish feeds include genistein, daidzein, bioachanin-a and coumestrol (Matsumoto et al. 2004). Phytoestrogens have been found to regulate fatty acid metabolism (D'Souza et al. 2005; Torno et al. 2019) in fishes, thus affecting tissue fatty acid content. The aim of the present study was the investigation of the effect of two diets with different phytoestrogen content on fillet and liver fatty acid composition of sea bream.

Materials and Methods

Two diets, A (protein 44.2%, fat 15.1%, ash 7.3% and moisture 6.4%) and B (protein 44.9%, fat 16.6%, ash 6,9% and moisture 6.5%), were provided by Biomar Hellenic. The phytoestrogen content of feeds was determined by LC/MS-MS. The compounds identified were daidzein (10.4±0.2μg/g for diet A and 2.64±0.06 μg/g for diet B), genistein (3.9±0.2μg/g for diet A and 1.4±0,1 μg/g for diet B), biochanin A (1.14±0.03μg/g for diet A), coumestrol (1.98±0.07 μg/g for diet A and 0.72±0.05μg/g for diet B) and glycitein (0.42±0.05μg/g for diet B). Specimen initial size was 101.4±0.8g. A 12-week experimental rearing was carried out in a Recirculating Aquaculture System (3 replicates/treatment, 12 fish/tank). Fish were fed at satiation twice a day. At the end of the experiment, fillet and liver samples were collected. Fatty acid composition of the tissues and the feeds was determined by GC/FID. Dietary fatty acid profile demonstrated no significant differences.

Results

No significant differences were observed between treatments for fillet and liver lipid content. High phytoestrogen treatment (Diet A) demonstrated significantly higher fillet SFA, liver n-3, n-3 LC PUFA, n-6, EPA and DHA content, than Diet B. On the other hand, Diet B demonstrated significantly higher fillet n-3, n-3 LC PUFA, EPA, DHA and liver MUFA content, that Diet A.

Discussion

Sea bream fatty acid content has been shown to reflect the respective content of the diet (Houston et al. 2017). Nevertheless, the similar dietary fatty acid profiles of the treatments point to the effect of the differences in phytoestrogen content. In the present study, the high phytoestrogen treatment, was characterized by an abundance of dietary daidzein and exhibited lower fillet n-3 LC PUFA content. This may be attributed to the regulatory effect of daidzein on fatty acid oxidation in muscle cells (Kitamura et al. 2020). Genistein on the other hand has been shown to increase DHA and n-3 LC PUFA content in the fillet of sea bream (Torno et al. 2019). This was not the case in the present study, pointing to a potential interaction of phytoestrogens, that needs to be clarified via specialized experimental design. On the other hand, the higher liver DHA and n-3 LC PUFA content was in accordance with data from the aforementioned study, indicating a potential tissue-specific prevalence of the different phytoestrogens. Additionally, biochanin-A (Park et al. 2016) and coumestrol (Zywno et al. 2021) have been shown to regulate hepatic fatty acid oxidation, thus contributing to the need to investigate the combined effects of phytoestrogens. Moreover, treatment A exhibited significantly lower GPT, cholesterol and triglyceride values, as well as a trend for lower LDH and GOT values (Tsopelakos et al. 2021), indicating the previously reported prophylactic effect of phytoestrogens on the liver (Park et al. 2016; Zywno et al. 2021). In conclusion, different dietary phytoestrogen content, affects significantly the fatty acid composition of fillet and liver. Nevertheless, further experimentation is required, in order to clarify the interaction between different phytoestrogens.

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GROWTH, BLOOD METABOLIC PARAMETERS AND GUT HEALTH STATUS IN RAINBOW TROUT (*Oncorhynchus mykiss*) FED FISH MEAL-FREE DIETS SUPPLEMENTED WITH CONVENTIONAL FEED ADDITIVES OR DRIED MICROBIAL BIOMASS AND RED SWAMP CRAYFISH MEAL AS FEED SUPPLEMENT

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Introduction

The readily available and cost-effective plant protein-rich ingredients have become the main protein sources used in aquafeeds to substitute unsustainable marine-derived ones. However, the transition to plant-based diets is more difficult to achieve for carnivorous fish, like salmonids, in which certain plant protein-rich ingredients can cause adverse effects on growth, liver integrity and gut health. In this regard, functional feeds represent an interesting solution to mitigate these drawbacks, even at low dietary inclusion. Conventional feed additives, like nucleotides and sodium butyrate, have been shown to positively modulate immune response by preserving intestinal integrity in salmonids. Furthermore, a new generation of more sustainable ingredients can be intended as feed supplements due to their promising features. The dried microbial biomass of certain microalgae and cyanobacteria represents a source of vitamins, minerals, carotenoids and antioxidant compounds. Differently, the meal obtained by the Louisiana red swamp crayfish (*Procambarus clarkii*), a highly invasive alien species in Europe, can be exploited as an innovative feed supplement converting an ecological problem to a natural source of protein and bioactive compounds such as astaxanthin and chitin. The present study was aimed at exploring the nutraceutical effects of low dietary inclusions of conventional feed additives (nucleotides and sodium butyrate) or innovative potential feed supplements such as Louisiana red swamp crayfish meal (RCM) and dried microbial biomass from intact cells of *Tetraselmis suecica* (TS) and *Artrhospira platensis* (spirulina; AP) in fish meal-free diets during a 104 day feeding trial performed on rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Four grossly isoproteic (42%), isolipidic (24%) and isoenergetic (23 MJ/kg) test diets were formulated starting from a basal one, named CV, characterized by a 10:90 ratio between marine (hydrolysed fish proteins used as feed stimulant) and vegetable protein sources. For conventional feed additives, a diet coined CV*plus* was prepared by adding sodium butyrate and nucleotides (0.2 and 0.05%, respectively) to CV. The test ingredients (RCM, TS and AP) were used to replace 10% of dietary crude protein supplied by the major plant protein-rich ingredients of the basal diet CV. A total of 750 rainbow trout (79.7 ± 13.5 g) were randomly distributed into 15 squared fiberglass tanks (1.6 m^3; 3 tanks per dietary treatment). At the end of the trial, zootechnical parameters were measured and samples of blood, liver and distal intestine were carefully collected and properly stored for the following analyses. (i) Histology: a series of histo-pathological indexes were evaluated to assess the gut health status, with emphasis on mucosal fold height and inflammatory influx, and the degree of fat accumulation in hepatic parenchyma. (ii) Fourier Transform Infrared Imaging (FTIRI): to evaluate the biochemical composition of liver tissue, false colour images were created resulting from integration process of IR maps in the spectral regions of lipid, protein, and glycogen components. Spectra were extracted and band area ratios were calculated and statistically analysed. (iii) Real-time PCR: relative quantification of the expression of genes involved in immune response (*tlr1*, *nfkb*, *il1b*, *il10*, and *tnfa*) was performed on distal intestine samples. (iv) Blood chemistry: serum aliquots were stored in dry ice, then metabolites, transaminases and minerals were analysed by spectrophotometric assays. (v) Metataxonomic analyses on intestinal microbiota: faecal content was collected, frozen in liquid nitrogen and then analysed by Illumina Miseq sequencing of V3-V4 region of 16S rRNA gene, using Qiime2 for data analysis.

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Results and Discussion

The provision of a diet rich in vegetal protein sources (CV) resulted in a severe impairment of distal intestine morphological and histopathological indexes, without affecting growth performances in relation to the best diets here compared. The CV*plus* diet did not result in a significant improvement of zootechnical parameters and showed only a marginal ameliorative effect on distal intestine health status that remained highly inflamed. Both AP and TS diets reduced microbiota diversity compared to the other groups but showed opposite results in terms of growth and gut. Despite no significant differences with CV and CV*plus* diets, fish fed AP diet showed improved zootechnical performances respect to those fed both RCM and TS dietary treatments, possibly due to an enhanced nutrient uptake and a higher digestible-nutrient intake as respectively evidenced by: (i) a significantly higher supranuclear vacuolization of distal intestine enterocyte; (ii) a higher nutrient digestibility of AP compared to that of TS, characterized by thick and cellulosic cell wall. Accordingly, fish fed TS diet were characterized by depressed growth performances compared to fish given CV, CV*plus* and AP diets and by a stronger selective pressure on microbiota, probably favouring those bacteria with specific cellulolytic activities. However, considering the distal intestine health status, providing TS diet resulted in a well-structured and more developed intestinal absorptive epithelium and led to a significant reduction of inflammatory influx respect to both CV and AP diets. In fact, the AP dietary inclusion was not able to fully contrast the negative side-effects of the vegetable ingredients on gut health, leading to a high degree of basal inflammatory influx. Finally, providing 10% dietary crude protein in the form of RCM did not affect zootechnical parameters compared to those observed for fish given diet CV, despite the high biogenic amines content could have led to a reduction in feed consumption and consequently in growth compared to CV*plus* and AP groups. On the other hand, RCM showed promising results due to its ameliorative effects on gut histological conditions, since it is a source of carotenoids (mainly astaxanthin) with a potential beneficial role on distal intestine. In addition, gut of fish fed RCM diet harboured high percentages of the Proteobacteria genus *Deefgea* (12%), a specific bacterial genus that provides a strong chitinolytic activity. Considering liver, both histological and spectroscopic analyses detected a low hepatic lipid accumulation in fish from all the dietary treatments. Based on blood chemistry analyses, the metabolic status, in terms of total proteins and lipids, were similar in all dietary groups, whereas a significant increasing of minerals content was observed in fish fed RCM, TS and AP diets.

As a conclusion, results from the present study demonstrated that both conventional feed additives and all the novel feed supplements investigated were able to ameliorate responses of rainbow trout when added to a vegetable-based diet.

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SEARCHING FOR A SUITABLE COMMERCIAL DIET FOR CARPIONE, *Salmo carpio*, TO SUSTAIN A PROPER GROWTH, WELFARE, AND FILLET QUALITY

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Introduction

Carpione (*Salmo carpio*) is a precious endemism of Lake Garda, particularly refined for its high-quality meat thus representing an important commercial resource for the local community. Initially very abundant, in the last three decades the wild population has been drastically reduced leading to the insertion of carpione in the IUCN list of severely endangered fish species. The possible extinction of this species would result in a biodiversity loss and in economic repercussions. On this regard, the farming of this species has been recognised as a solution to ensure the fish product and thus maintaining the gastronomic tradition. However, little is known about the natural diet of carpione raising a concrete issue for the farmers. Currently, a specific diet has not been yet formulated for the growth-out phase leading to the use of diets intended for other salmonids basing the choice on the best compromise between cost and benefit instead of the nutritional requirements of carpione. For that reason, the aim of the present study was to test four diets in which the protein fraction derived from different origins and evaluate their effects on growth, welfare and fillet biochemical composition of carpione during a 3-month feeding trial.

Materials and Methods

Four isolipidic, isoproteic and isoenergetic diets were formulated. Specifically, a diet in which the protein fraction was mainly constituted by marine-derived ingredients (60%) was used as control (CTRL). The other three diets were formulated by partially replacing marine-derived ingredients with plants-derived ones (70% of the total protein fraction; VEG diet) or with land animal proteins (56 or 68 % of the total protein fraction, named PAP1 and PAP2, respectively). Fish were divided in twelve independent fiberglass tanks (700 L; three tanks per dietary group; 40 fish each) and the diets were administered *ab libitum*, 6 days a week, over the whole feeding trial. The fish physiological responses to each dietary treatment were assessed through a multidisciplinary approach including zootechnical performances, distal intestine and liver histology, gene expression of markers involved in immune and stress response (on distal intestine and liver samples, respectively), physical and chemical analyses of the fillet, and macromolecular composition of liver and muscle samples through spectroscopic analyses (FTIR).

Results and Discussion

Fish from each dietary treatment did not show significant differences in terms of growth performance and feed conversion ratio. However, fish welfare was affected by the dietary inclusion of plants-derived ingredients. In fact, an increase in inflammatory influx (including abnormal presence of melanomacrophages), along with the upregulation of all the inflammatory gene markers analysed on distal intestine samples (*il6*, *il10* and *tnf-α*), were highlighted in fish fed VEG diet. Conversely, CTRL diet did not impair the distal intestine health status, while PAPs diets resulted in a better gut condition compared to the others, consisting in a reduction of inflammatory influx and a significant modulation of inflammatory gene expression. Moreover, the enterocytes supranuclear vacuolization increase in distal intestine from PAPs groups indicates a further improvement in gut functionality in fish from these groups. On the other hand, liver composition was differently affected by the diets tested in the present study. Particularly, while CTRL and VEG groups showed a low degree of lipid deposition in hepatic parenchyma, PAPs inclusion caused a higher, dose-dependent, lipid accumulation in liver tissue, along with a higher amount of total fat in the fillet (as detected by both chemical and spectroscopic -FTIR- analysis), and a consequent *hsp70* upregulation, even if not significant, in liver samples. Since diets were formulated to be grossly isolipidic, these differences can be attributed to the dietary fatty acids composition. Specifically, PAP2 diet showed a higher amount of monounsaturated fatty acids and a higher n6/n3 ratio compared to CTRL and VEG ones, which can be related to the hepatic steatosis onset and the high percentage of fat in the fillet. Moreover, while VEG diet marginally affected fillet fatty acids profile compared to CTRL one, a decrease in EPA (20:5n3) and DHA (22:6n3) content was observed in fillets from PAP1 and, particularly, in PAP2 compared to those from CTRL, consistently with the lower amount of these fatty acids in the corresponding diets. This scenario highlighted a reduced ability of carpione in synthesise long-chain

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polyunsaturated fatty acids starting from shorter chain precursors. Finally, the present work revealed a marginal effect of the tested diets on the other quality parameters analysed. Particularly, considering skin colour, the presence of PAPs at different levels induced a discoloration of fish skin respect to CTRL and VEG groups, while the hardness of the fillets was increased in VEG and PAP2 fillets.

In conclusion, the provision of vegetable-derived ingredients affects the gut welfare of carpione comparably to other salmonids fish species, while marine-derived ones (fish meal and fish oil) have been demonstrated to act as adjuvant in counteracting these negative side effects. On the contrary, PAPs used in the present study were able to promote fish welfare by promoting gut health and absorption capacity. However, their use must be further optimized to guarantee an adequate fillet fatty acids profile to achieve the quality traits and the consumers acceptance of the final product.

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EFFECTS OF GARLIC *Allium sativum* POWDER ON CROWDING STRESS RESPONSE IN EURASIAN PERCH *Perca fluviatilis* JUVENILES

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Introduction

Medicinal plants have been used in fish diets as natural antistress (Adineh et al., 2020). Garlic (*Allium sativum*) is a medicinal plant that belongs to the *Liliaceae* family (Talpur et al., 2012). Eurasian perch (*Perca fluviatilis*) is a newly domesticated species that demonstrated potential for European inland culture last years (Policar et al 2015). The present study aimed to evaluate the effects of garlic powder in feed on resistance to crowding stress in Eurasian perch juveniles.

Materials and Methods

Perch juvenile was adapted for 14-days in a recirculation aquaculture system (RAS), and 1320 juvenile perch were distributed in twelve 185 L tanks (110 fish per tank). Feed was extruded at Exot Hobby s.r.o. (Černá v Pošumaví, Czech Republic). Fish with an initial weight of 25.0±0.4 g were fed a diet including 0 (Control), 10 (G10), 20 (G20), and 30 (G30) g kg^{-1} garlic powder manually based on apparent satiation three times a day at 08.00, 12.00, and 16.00 for 87 days. At the end of the feeding trial, fish were crowded with water level descent, netted, and removed from the water for 30 s, and were then returned to the tank, where the water level of the tank was increased back to the original volume, and the density was reduced. Two fish per tank were randomly selected before stress (Pre-stress), immediately post-stress time 0, time 1, time 6, and time 24 h post-stress. (n = 6 per group).

Results and Discussion

Pre-stress, no significant difference in the level of cortisol among groups was observed ($p > 0.05$). At time 0, the level of cortisol was significantly higher in all garlic groups compared to the control ($p < 0.05$). At time 1, the lowest level of cortisol was observed in G30 significantly compared to all groups ($p < 0.05$). At time 6, G20 showed the lowest level of cortisol among groups significantly ($p < 0.05$). At time 24, significantly lower level of cortisol was detected in all garlic groups compared to the control ($p < 0.05$). The glucose levels in pre-stress and time 0 showed no significant difference among groups ($p > 0.05$). At time 1, glucose levels significantly decreased in G20 and G30 compared to the control and G10. At time 6, levels of glucose decreased in G30 compared to the other groups significantly. At time 24, all groups fed with garlic showed significantly lower level of glucose compared to the control. Garlic powder at levels 5, 10, 15, and 20 g kg^{-1} in the feed of Asian sea bass decreased the level of blood serum glucose (Talpur et al., 2012) significantly, and it can be related to the garlic organosulfur compounds such as alliin (S-allyl cysteine sulfoxide) (Zhai et al., 2018). The use of 2 mg nano selenium and 2 ppm garlic extract decreased blood plasma cortisol and glucose in grass carp (*Ctenopharyngodon idella*) under crowding stress (Adineh et al., 2020). Decreasing the level of glucose in the garlic group can be attributed to the bioactive compounds of garlic including diallyl trisulfide (Han et al., 1995), which were higher in the G30 diet compared to the other diets (Talpur et al., 2012). The present study showed that garlic powder at level 30 g kg^{-1} (G30), can decrease the level of cortisol and glucose one hour after crowded stress compared to control significantly.

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GROWTH PERFORMANCE OF JUVENILE AND PRE-ADULT SOBAITY SEABREAM *Sparidentex hasta* FED WITH FORMULATED IMPROVED FEED UNDER THE RED SEA CONDITIONS

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Introduction

Sobaity seabream (*Sparidentex hasta*) popularly known as silvery-black porgy, is a commercially important carnivorous fish species in the Arabian Gulf sea region (Mozanzadeh et al., 2017). Knowing that this fish is native from the Red Sea, its suitability to the existing environmental conditions (salinity: 41-42‰; optimum temperature 32°C, based on our earlier research) makes this species a good candidate for aquaculture in the country as well as in the Middle East region. One of the major challenges in aquaculture of this species is to develop less wasteful and economic diets. The commercial success in aquaculture operation of the candidate aquatic species will partially depend on the determination of its optimum feed formulation and feeding schedules during the grow-out. In view of the above, this study was conducted to evaluate the performance of practical formulated feeds in comparison to commercial feed for the juvenile (experiment I) and pre-adult stage (experiment II) of Sobaity seabream *Sparidentex hasta*.

Materials and Methods

In this study, two baseline experiments were conducted for 74 days from the Red Sea (41-42‰ salinity, 24.7-30.7°C temperature) to evaluate the performance of practical formulated feeds in comparison to commercial feed for the juvenile (experiment I) and pre-adult stage (experiment II) of Sobaity seabream *Sparidentex hasta*. The fish (195.22 ± 5.8g for experiment I; 298.6± 18.1g for experiment II) was randomly distributed in triplicates of 450-L circular tanks supplied with filtered seawater from the Red Sea. Two test diets D1 and D2 containing 55 and 51% crude protein, 16.9 and 15.8% crude lipid, respectively, were formulated. Additionally, a commercial diet (Diet_{com} 46% crude protein) was used as a control diet. In experiment I, D1 and D_{com} were compared and in experiment II, D1, D2 and D_{com} were compared. In both experiments, triplicates were hand-fed three times a day.

Results

In experiment I, D1 group showed better growth performance (334.6 g over the 74 days feeding trial) and FCR (1.68) than Diet_{com} group (302.62 g, 2.19 FCR). Fish fed D1 diet showed 10.6% gain in weight than that of commercial diet. The better chemical score (CS) of histidine, arginine, threonine, valine, methionine, lysine, isoleucine and essential amino acid index (EAAI) was found in fish fed D1. In experiment II, the maximum growth performance (538.83 g) and lowest feed conversion ratio (1.69, Fig. 1) was noted in fish fed diet D1. Fish fed D1 diet exhibited 10.1% increase in weight in comparison to commercial diet. The CS of histidine, arginine, threonine, valine, methionine, lysine, isoleucine, leucine, phenylalanine and EAAI was found to be best in fish fed D1. A simple economic analysis exhibited that the feed cost per kilogram of fish production was significantly ($P < 0.05$) lowest in diet D1 (USD 3.18 in experiment I, 2.43 in experiment II) followed by the Diet_{com} (USD 3.39 in experiment I, USD 2.56 in experiment II) and then D2 (USD2.7 in experiment II).

Discussion

In experiment I, Sobaity seabreams fed fish meal based diet D1 presented a higher growth performance compared to fish fed commercial diet, while in experiment 2 the partial replacement of fish meal by plant-based protein in D2 resulted in lower fish performance in terms of body weight compared to D1 and commercial diets regardless the dietary protein contents. This reduction in growth performance may be due to low palatability, protein digestibility, availability of essential amino acid and minerals, and also because of the presence of the anti-nutritional factors (Trushenski et al., 2014; García-Ortega et al., 2015). The reduction in the fish growth of D2 treatment with response to the replacement of dietary fish meal by high inclusion of dietary plant proteins has also been published in other aquatic organisms (NRC 2011).

Conclusions

Based on the results, D1 (55% CP) is recommended for improving the culture of sobaity in the Kingdom of Saudi Arabia.

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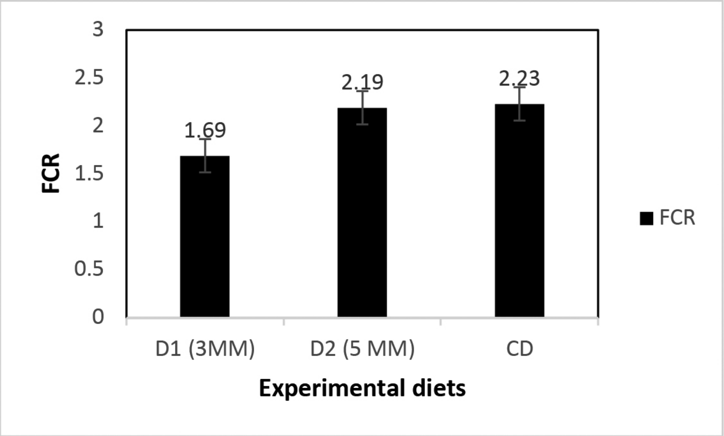

Fig. 1. FCR of preadult sobaity seabream fed improved (D1, D2) and commercial diets (CD).

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GROWTH PERFORMANCE, PROXIMATE COMPOSITION, AND FEED CONVERSION RATIO OF JUVENILES AND PRE-ADULT GILT HEAD SEABREAM (*Sparus aurata*) FED COST-EFFECTIVE, IMPROVED DIETS

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Introduction

Emerging trends in mariculture, the countries having long coastal shoreline improving the aquaculture industry for culturing different marine carnivorous fishes in marine cages. Gilthead seabream (*Sparus aurata*) is one of the most cultured species in cage nets in the Kingdom of Saudi Arabia for more than four decades (De Francesco, 2014). Aquaculture of this species requires cost-effective, nutritionally-balanced feeds. In view of the above, this study was conducted to evaluate the effect of cost-effective improved diets (ID3 and ID5) and commercial diets (CD) on growth performance, proximate composition of juvenile and pre-adult gilthead seabream (*Sparus aurata*).

Materials and Methods

Two different formulations of ID3 and ID5 with a crude protein content of 50.4% and 48.2% were used for juvenile and preadult, respectively. Additionally, commercial diet (CD with 50.7% crude protein) was also used to compare the performance of improved diets for juvenile and pre-adult stages of gilthead seabream.

Results

Feed conversion ratio (Fig. 1), total feed intake, protein-energy ratio, proximate protein content, and protein retention were significantly higher in juvenile fish fed ID3 than in fish fed CD. No significant differences were found in specific growth rate, condition factor, lipid, and energy retention between fish fed ID3 and CD diets. In the pre-adult gilthead seabream, no significant differences were observed in feed conversion ratio, total feed intake, specific growth rate, protein-energy ratio, proximate composition, protein, lipid and energy retention between fish fed ID5 and CD diets. This study indicates that dietary protein level of 50.4% and 48.2% can improve the growth performance of juvenile and pre-adult fish, respectively. The economic conversion efficiency for the improved diets (ID3 and ID5) in juvenile and preadult was found to be lower than that of commercial diets.

Discussion

The crude protein content of the formulated diets (ID3 and ID5) were higher compared to commercial diets and also higher than dietary crude protein reported previously in the literatures (Guerreiro et al., 2019).

The specific growth rate values (1.21 to 1.23%) obtained with the juvenile gilthead seabream in the present study were higher than those SGR values (0.89 to 1.00%) with the juvenile gilthead seabream reported by Sanchez-Moya et al. (2020). Feed conversion ratio of juvenile gilthead seabream fed ID3 was significantly lower as compared to the fish fed commercial diet (CD).

Previous studies suggested that feed that contains high protein (>49%) and high lipid content (15-22%) results in better FCR (0.94-1.12) and good growth performance (Emre et al. 2013).

Conclusions

Since economic conversion efficiency was lower for ID3 (juvenile) and ID5 (preadult) than the commercial diets, diet ID3 (50.4% protein) and ID5 (48.2% protein) may be recommended for the culture of juvenile and preadult of gilthead seabream, respectively.

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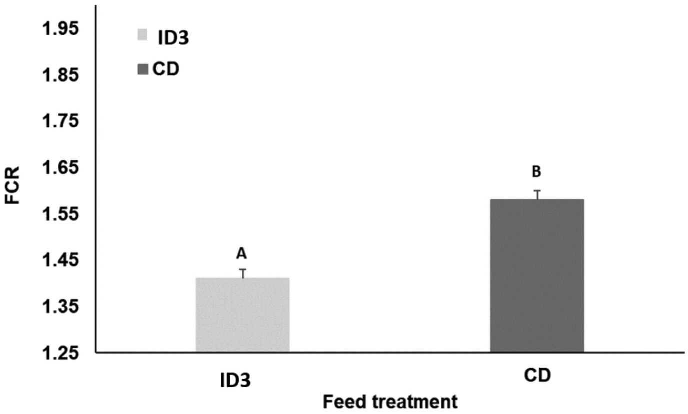

Fig. 1. FCR of juvenile gilthead seabream fed improved diet (ID3) and commercial diet (CD).

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HAEMATOLOGICAL RESPONSES AND HISTOLOGICAL EVALUATION IN LIVER, SKIN AND GILLS OF FARMED EUROPEAN SEA BASS; *Dicentrarchus labrax* EXPOSED TO ULTRASOUND

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Introduction

Biofouling is one of the major problems faced by cage aquaculture farmers worldwide, and its management (costs of chemicals, nets cleaning and repair, and fish escapees) is expensive. Current antifouling practices aimed at preventing or minimizing biofouling growth are predominantly achieved through the use of antifouling agents such as paints and coatings. However, these antifoulants contain toxic substances, which can adversely affect marine organisms and their environment. Therefore, research into alternative environmentally friendly antifouling systems such as the application of ultrasound (US) in marine aquaculture is essential. However, the impact of US on non-target aquatic species, such as commercially important farmed fish, in aquaculture facilities has yet to be extensively evaluated. Therefore, the present study explored and investigated fish cage net cleaning using a US device and its effect on the high-value fishery and aquaculture species European sea bass, *Dicentrarchus labrax* after 12 weeks exposure under commercial culture conditions in Turkey. The effects of applied US on haematology and histology in sea bass liver, skin and gills were evaluated. In addition, feed intake, growth parameters, hepatosomatic index, health and immune responses were monitored.

Material and Methods

A field trial was conducted in a commercial offshore aquaculture fish farm in Izmir, Turkey for 12 weeks. European sea bass juveniles with an initial weight of 49.95±13.30 were raised in operational cages (two test and two control cages; diameter: 30 m, depth: 20 m, stocking density: >50.000). The four cages used in this trial were not protected against fouling (no anti-fouling coating was used) to enable the evaluation of the postulated antifouling effect of the US system installed as a prevention measure in two of the cages (Test (T) cages: T1 and T2) against two untreated cages (C1 and C2) used as control (C). Test cages equipped with US devices were set-up for a fixed range frequency (22kHz +/- 100Hz and 35kHz +/- 100Hz) and operational mode full fire. The impact on farmed sea bass in offshore cages was examined throughout sequential sampling including a reference sampling (d0) before ultrasound system (US) application, after one week (d7), and further in a one-month interval (d37, d67 and d97) of continuous application. Fish behavior (swimming, feed intake) were monitored every day by the fish farm. A total of 200 European sea bass (10 fish/cage) were sampled for further laboratory analyses.

Results and Discussion

This study concluded that the growth and survival of analyzed European seabass in T and C groups were unaffected by a long-term US exposure under aquaculture conditions. Furthermore, histological examination of the liver hepatic parenchyma did not present any distinguishable histopathologic abnormalities or changes attributable to US between T and C groups. A comparison of the results of the blood smears (red and white blood cell, and trombocytes counts) before and after exposure to US did not reveal any major changes, as the counts of the blood cell types of both the first sampling (d0) and the last sampling (d97) were in matching ranges. The application of US as an antifouling method in mariculture systems is practicable and does not cause any noticeable health restrictions in European sea bass (Dicentrarchus labrax), at least at a frequency range of 22kHz - 35kHz (± 100Hz) over a trial period of 3 months. Nevertheless, final histological evaluation of sea bass skin and gills are pending!

As stress response can differ between species, the end product should be tested in other commercially important species as well in order to proof the safeness of system and tune fine it for each species and condition.

This study is part of the EU-project “Smart System for the Prevention of Biofouling on Aquaculture NETs by Ultrasonic Wave Technology” (NetWave), and it has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 958776.

EFFECTS OF ASTAXANTHIN FROM CORYNEBACTERIUM GLUTAMICUM ON GROWTH PERFORMANCE, MUSCLE PIGMENTATION AND HEALTH STATUS OF JUVENILE RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

Colouration of fish, especially salmonids, is an important quality criterion for consumers. In nature, salmonid fishes get their muscle colouration through their natural prey, such as small crustaceans, which normally contain Astaxanthin (Asx). In aquaculture, the colouration can be achieved by supplementing Asx to the formulated diets. Asx can be produced synthetically or through natural vectors, such as microalgae or bacteria. Asx is not only responsible for the coloration of the fish, but can also have impacts on the antioxidant activity of fish (Sheikhzadeh et al. 2012). Therefore, in this trial, we investigated the effects of Asx from *Corynebacterium glutamicum* and compared it with other sources of Asx on growth, muscle pigmentation and health status in juvenile rainbow trout.

Material and Methods

Feed enriched with Asx from different sources was investigated in juvenile rainbow trout with an initial weight of 25 ± 3.6 gr. Five feed groups were examined in quadruplicates (C: without Asx supplement (control); SA30: synthetic Asx; PA30: natural Asx supplement, Panaferd©; AA30: algae-based Asx, *Haematococcus pluvialis*; BA30: bacteria-based Asx, *C. glutamicum*). Forthy fish per replicate were fed for 8 weeks and growth was monitored at experiment onset, after four weeks and at the termination. Ten fish per replicate were sampled in the end of the experiment for muscle and liver Asx content determinationt by HPLC. Fillet colour were determined visually by DSM SalmoFan card scores. In addition, whole body composition, organ indices and haematological responses were assessed.

Results and Discussion

Final Results are pending. However, the first results from mid-term sampling (after 4 weeks) showed clear differences on growth and pigmentation of fillets of rainbow trout fed with different Asx source supplement diets. Although the pigmentation of bacteria-based Asx fed groups were very promising as other Asx source fed groups, the growth was lower than other experimental groups. However, conclusions are not possible since the full data set is not available yet. Preliminary results are presented in Table.1 and Figure. 1.

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Table I. Mean body weight ($BW_{\text{mid-term}}$), weight gain ($WG_{\text{mid-term}}$), mean length and length gain ($LG_{\text{mid-term}}$) of rainbow trouts fed with diets C: without Asx supplement (control); SA30: synthetic Asx; PA30: natural Asx supplement, Panaferd©; AA30: algae-based Asx, Haematococcus pluvialis; BA30: bacteria-based Asx, C. glutamicum) for 4 weeks at inclusion rates of 30mg/kg each.

Experimental diets	**$BW_{\text{mid-term}}$ (g)**	**$WG_{\text{mid-term}}$ (g)**	**Length $_{\text{mid-term}}$ (cm)**	**$LG_{\text{mid-term}}$ (cm)**
C	68,63 ± 12,9	43,66 ± 9,3	17,8 ± 1,5	4,69 ± 0,76
SA30	74,97 ± 12,2	49,09 ± 8,5	18,2 ± 1,4	4,97 ± 0,44
PA30	71,08 ± 7,8	46,12 ± 4,3	18 ± 0,8	4,93 ± 0,01
AA30	65,49 ± 11,7	40,58 ± 7,9	17,3 ± 0,9	4,24 ± 0,23
BA30	41,66 ± 6.3	16,82 ± 2,7	15,6 ± 0,8	2,56 ± 0,16

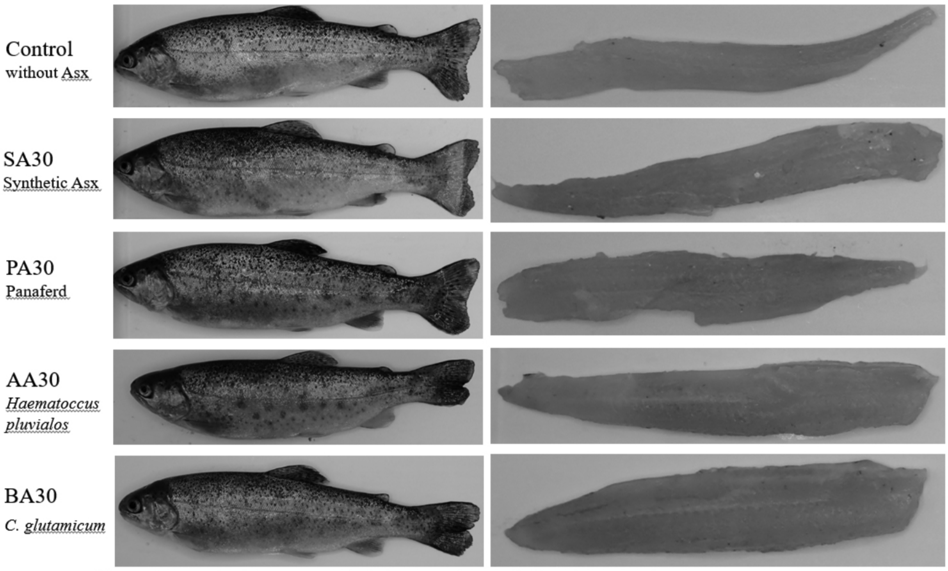

Figure 1. Fillet colours of rainbow trout fed the experimental diets for 4 weeks.

EXPERIMENTAL RESULTS ON THE LIGHT AND TEMPERATURE REQUIREMENTS OF FURCELLARIA LUMBRICALIS (HUDSON) J.V.LAMOUROUXF FROM THE PUCK LAGOON (SOUTHERN BALTIC SEA)

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Introduction

Clawed Fork Weed *Furcellaria lumbricalis* (Hudson) J.V.Lamouroux is a species native to the waters of the Baltic Sea, which formed rich communities on the bottom of the Puck Lagoon (Southern Baltic) until the 1960s. As a result of severe anthropopressure from exploitation by dredging, rapidly increasing levels of eutrophication and industrial pollution, between 1970 and 1980, the lagoon's environment was severely degraded, which ultimately led to the disappearance of the weed population. *F. lumbricalis* has been observed again in the waters of the lagoon since 2019 and the population is gradually increasing its range.

Due to the fact that this taxon has a very high commercial potential and is exploited in another area of the Baltic (Estonia) any information on the biology and ecology of the taxon is extremely valuable. In this study, we will present the results of an experiment to explore the ecological requirements of the current *F. lumbricalis* population in relation to temperature and salinity in the context of biomass growth.

Materials and methods

F. lumbricalis was collected from a beach near the waterline in June and October 2021 in the vicinity of Rewa (54°40'18.18 "N; 18°32'19.39 "E). After being transported to the laboratory, the tissues were cleaned of overgrowing plant and animal organisms and organic and inorganic particles. Only undamaged specimens, visually in the best possible condition, were selected for the experiments. Before starting the experiment, the initial weight of specimens was determined, after draining excess water. The experiment was carried out on plants acclimatised to the experimental conditions for a period of 7 days. The study was carried out in an aquarium flow-through system, consisting of three circuits with four aquaria in each (tank dimensions: 50 x 35 x 30 cm; volume: 50 l).

Light of four, different intensities of photon flux density was used for the experiment, i.e.: 50, 110, 170 and 200 μmol photons m^{-2} s^{-1} and three water temperature values, i.e.: 10, 15 and 20 °C. Each experimental treatment was performed in three independent replicates by placing 2 dm^3 glass tanks inside each aquarium. Three to four specimens of about 2.91 g m.m were placed in each tank.

The plants were maintained in a 1.5 l culture solution with a salinity of 7 PSU, prepared on the basis of artificial Marine seawater with the addition of nutrient solution. The experiment lasted for a total of 50 days and biomass measurements were taken when the culture medium was changed, i.e. every 7 days. Based on the results of the biomass measurements, the Daily Growth Rate (DGR) was determined.

Results

At 10 °C, the DGR rate varied from -1.59 % to 3.58 % on average. The highest values were observed on the 15th day of measurements (from 1.80 % for light of 50 μmol photons m^{-2} s^{-1} to 3.58 % for light - 170 μmol photons m^{-2} s^{-1}), while negative values of the index occurred on the 22nd day after the start of the experiment. Considering the magnitude of the light, the highest values of the DGR rate occurred in high light of 200 μmol photons m^{-2} s^{-1}, even on the last day of the test, despite the high necrosis of the fronds, the DGR was 1.0 %. On the last day of measurement, only at light 110 and 50 μmol photons m^{-2} s^{-1} the DGR index had a negative value.

At 15 °C, the DGR index ranged from -4.19 % to 4.58 %, with the highest values on the 15th day of measurement (from 1.63 % for a light of 50 μmol photons m^{-2} s^{-1} to 4.58 % for a light of 170 μmol photons m^{-2} s^{-1}). Negative values of the index occurred after the 22nd day of the experiment, at which time fragmentation of the fronds was observed. Considering the light intensity, *F. lumbricalis* had the highest growth values falling mainly on the 15th day of the test, with subsequent measurements, positive values of the DGR indicator were observed mainly in the low light of 50 μmol photons m^{-2} s^{-1}.

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At 20 °C, the DGR ratio ranged from -12.98 % to 5.41 %. The highest values were observed on the 15th day of measurement (1.82 % for light of 50 μmol photons m^{-2} s^{-1} to 5.41 % for light of 200 μmol photons m^{-2} s^{-1}), and negative values of the index were observed 22 days after the start of the experiment. It was then that strong necrosis and decay of the fronds was observed. After day 29 of the experiment, there was a strong loss of biomass in each light variant.

In all temperature variants (10, 15 and 20 °C), maximum DGR values occurred at aquaria with high light (170-200 μmol photons m^{-2} s^{-1}), and minimum values occurred at low light (50 μmol photons m^{-2} s^{-1}). The first fronds decomposition and the successive decrease in biomass always occurred between days 15 and 22 of the experiment. The lowest DGR rates were determined for *F. lumbricalis* in circulation with a water temperature of 20 °C.

Conclusions

The growth of *F. lumbricalis* was most influenced by temperature, followed by light. The most optimal conditions for growth with respect to water temperature were in the range of 10-15 °C, and for light in the range of 170-200 μmol photons m^{-2} s^{-1}. Maintaining cultures at high temperature combined with high light led to high fragmentation and necrosis of the fronds.

The use of artificial seawater for laboratory cultures and the treatment of *F. lumbricalis* prior to experiments was shown to be an insufficient way to avoid overgrowth of organisms and culture tanks by unwanted organisms.

Acknowledgement

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DETERMINATION OF THE OXIDATIVE STRESS BIOMARKERS OF 8-HYDROXYDEOXYGUANOSINE AND DITYROSINE IN GILLS, SKIN, DORSAL FIN, AND LIVER TISSUE OF ATLANTIC SALMON (*Salmo salar*) PARR

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Introduction

8-Hydroxydeoxyguanosine (8OHDG) and dityrosine (DIY) are specific biomarkers of DNA and protein damage from oxidative stress, respectively (Valavanidis, et al. 2009; Malencik, et al. 2003). Nowadays, 8OHDG analysis in fish is mostly performed with these specific kits/antibody methods (Oğuz, et al. 2018; Gyimah, et al. 2020; Alak, et al. 2017). However, the selectivity of ELISA in trace concentrations can be compromised from cross-reactivity with other co-occurring analogues, while simultaneous determination of 8OHDG and DIY with a single assay is currently not available. With this background, an extraction methodology tailored to UPLC®–ESI (electrospray)–MS/MS analysis was developed in the present study for the simultaneous determination of 8OHDG and DIY and was applied in tissue samples (skin, gills, dorsal fin, and liver) that were collected from Atlantic salmon parr individuals reared in an experimental recirculating aquaculture system (RAS) treated with peracetic acid.

Materials and Methods

The skin, gills, dorsal fin and liver were collected from 234 Atlantic salmon parr (4 x 234 = 936 samples) at the Tromsø Aquaculture Research Station in Kårvik, Norway (Mota, et al. 2022). The tissue samples were thawed in room temperature and a portion of ~100 mg of each tissue sample was transferred into a 15 mL PP tube. Samples were fortified with 15 µL of 1000 ng/mL isotope labeled IS-mixture followed by the addition of 600 µL MeOH containing 1% ammonium formate (w/v). Thereafter, the samples were vortex mixed (30 s) and ultrasonication (30 min) was performed followed by centrifugation (5 min, 3500 rpm). The supernatant was collected and transferred into a new 15 mL PP tube. Two different cleaning methods were tailored to the extraction protocol. Finally, the extarct was transferred for UPLC®-MS/MS analysis.

Results

The relative recoveries of 8OHDG and DIY were 101±11.1 % and 104±12.0 %, respectively, ensuring the accuracy of the extraction and quantification. The inter-day precision (method reproducibility, RSD %, N = 18) at the fortified concentration of 10 ng/mL were 5.62 and 5.18 % for 8OHDG and DIY, respectively. The chromatographic separation was carried out using a gradient elution program with a total run time of 5 min. The limits of detection (LODs) were 0.11 and 1.37 ng/g wet weight (w.w.) for 8OHDG and DIY, respectively. To demonstrate the applicability of the developed method, it was applied in 907 tissue samples that were collected from Atlantic salmon parr individuals reared in an experimental land-based recirculating aquaculturesystem (RAS) treated with peracetic acid. The detection rates of both target analytes were > 70% in the tissues, except from the low detection rate of 8OHDG reported in the liver samples (24%). Moreover, the possibility of using dorsal fin as an alternative matrix for the minimally invasive assessment of oxidative stress in Atlantic salmon parr was introduced.

Conclusions

A methodology tailored to UPLC®-MS/MS was developed for the determination of two oxidative stress biomarkers, 8OHDG and DIY, in gills, skin, dorsal fin, and liver tissue of Atlantic salmon parr. The method was applied successfully in 907 fish tissue samples from Atlantic salmon, and both biomarkers were detected in most samples. Moreover, correlations of the biomarkers across tissues were uncovered, introducing the possibility of using dorsal fin as an alternative matrix for the minimally invasive assessment of oxidate stress in Atlantic salmon parr.

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EFFECT OF EMULSIFIERS ON PERFORMANCE AND DIGESTIBILITY IN NILE TILAPIA FED HIGH OR LOW NSP DIETS

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Introduction

For the rapid expansion of the aquaculture sector, the feed industry is using amongst others plant ingredients as alternatives to replace fishmeal and -oil (FAO, 2016). However, the inclusion of low-quality plant ingredients as fishmeal alternatives will mostly result in decreased digestibility of these diets, as most plant ingredients contain anti-nutritional factors (Sinha et al., 2011). Shifting to plant-based feed with good nutrient digestibility is of importance for the aquafeed industry and long-term sustainability of the aquaculture sector.

Dietary fat is the most energy-dense macronutrient (proximately 39.5 kJ/g) in feed to fulfil the energy and fatty acid requirements of animals for maintenance and growth (Hardy & Kaushik, 2021). For good fat digestion processes, proper emulsification in the gastrointestinal tract needs to take place, whereby bile acids play a role. Fishmeal contains bile acids, while plant ingredients lack these bile acids, especially plant ingredients with high non-starch polysaccharide (NSP) contents. NSPs are far less digestible compared to fishmeal and also interferes with other feed macronutrients, overall resulting in decreased nutrient digestibility (Sinha et al., 2011). In addition, NSP are believed to enhance bile-acid losses, due to binding with faces, more faces production and deconjugation by the gut microbiome (Staessen et al., 2021). Therefore diets containing high NSP level are usually considered as low-quality diets. Replacing fishmeal with low-quality plant ingredients may therefore hamper fat digestion. It is hypothesized that dietary supplementation with emulsifiers can improve (fat) digestion in fish, especially for diets lacking fishmeal and being high in NSP content. This study assesses the potential to improve digestibility and performance of Nile tilapia by the supplementation of two types of emulsifiers (bile acid or a commercial emulsifier), which was tested with two types of diets; a diet with a low NSP content and a diet with a high NSP content.

Materials and methods

This experiment involved a six-week growth trial with Nile tilapia (*Oreochromis niloticus*). Six diets were tested in a 2 x 3 factorial design with 3 triplicates. The first factor is the quality of the diet, created by differences in NSP level (low versus high NSP level). The second factor is 3 types of emulsifier supplementation (Non; 0.5g/kg bile acid ; 0.5g/kg commercial emulsifier). The commercial emulsifier, Excential Energy Plus which is composed of 21% ethoxylated castor oil, was provided by Orffa (orffa.com). In total six diets were tested: Diet 1: Low NSP, without supplement; Diet 2: Low NSP + bile acids; Diet 3: Low NSP + commercial emulsifier; Diet 4: High NSP, without supplement; Diet 5: High NSP + bile acid; Diet 6: High NSP + commercial emulsifier. During the growth trial fish (average initial weight 48.37 ± 0.63 gram) were fed twice daily at apparent satiation. Faeces were collected during the last week of the experiment, to study nutrient digestibility.

Results

Our results showed a significant effect of NSP levels, but no effect of emulsifier on fish performance. Dry matter, fat and carbohydrate digestibility were all affected by the interaction of NSP levels and emulsifier supplement (Table 1). Fat ADC was the most strongly affected by emulsifier supplement and the interaction (Figure 1). In fish fed the low NSP diet, both bile acid and commercial emulsifier improved fat ADC, but no effect was found for the high NSP diet. These results indicate that the supplementation of bile acid/emulsifier can improve fat digestibility in Nile tilapia, but the effect seems to be dependent on the diet quality.

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Table 1. Effect of NSP levels and emulsifier supplements on apparent nutrient digestibility (ADC) of dry matter (DM), crude protein (CP), fat, energy and carbohydrate (Carb) and ash in Nile tilapia.

ADC %	**Low NSP**			**High NSP**				***P-value***		
	No supp	**Bile acid**	**Emulsifier**	**No supp**	**Bile acid**	**Emulsifier**	**SEM**	***NSP***	***Supp***	***NSP * Supp***
DM	74.60a	76.46a	76.43a	64.72b	63.80b	64.01b	0.41	<0.001	0.373	0.009
CP	92.73	93.18	93.14	89.52	89.21	89.52	0.16	<0.001	0.453	0.104
Fat	86.81b	88.55c	88.64c	83.85a	82.50a	83.78a	0.32	<0.001	0.04	0.001
Energy	79.20b	80.91b	80.73b	69.75a	68.61a	69.02a	0.37	<0.001	0.563	0.006
Carb	62.01b	65.14b	64.80b	48.32a	47.25a	47.40a	0.78	<0.001	0.372	0.037
Ash	40.37a	43.38a	44.35a	42.23a	40.59a	40.76a	0.91	0.064	0.41	0.023
P	56.29b	58.78b	58.91b	50.36a	49.60a	49.60a	0.59	<0.001	0.245	0.022
Ca	8.38	11.63	15.11	16.69	13.87	13.82	2.44	0.147	0.697	0.181

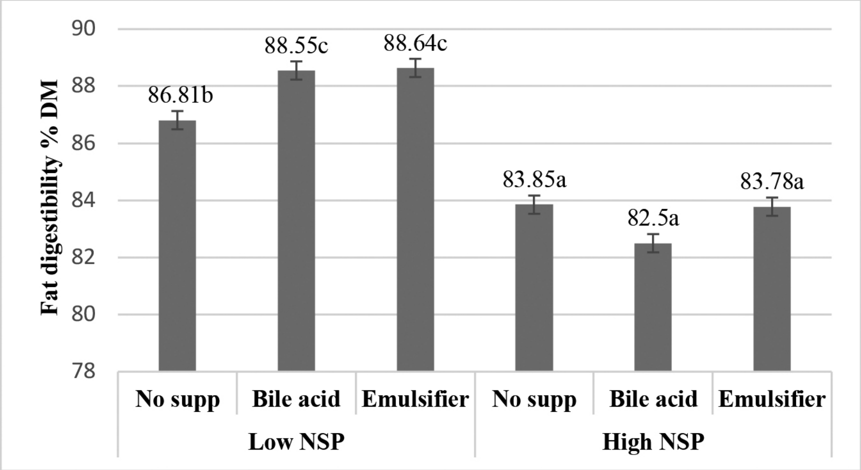

Figure 1. Effect of emulsifier supplement on the fat apparent nutrient digestibility (ADC) in Nile tilapia.

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CARBON BALANCE IN ZERO WASTE COUPLED AQUAPONICS WITH ONSITE ANAEROBIC TREATMENT UNDER THE DESERT CONDITION

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Introduction

Aquaponics (integration of aquaculture and hydroponics) is gaining renewed interest in enhancing food security. Several limitations, including energy and resource demand, nutrient imbalances, and waste management, have, however, limited their widespread application (Goddek et al., 2019; Somerville et al., 2014). Efficient onsite treatment of aquaponics waste and its conversion to nutrient and energy sources may be advantageous. Zhu et al. (2021) demonstrated that plant wastes (e.g., roots and inedible parts) from aquaponics could be treated with high efficiency using anaerobic digestion (AD). Similarly, this is also the case for AD of fish sludge from RAS (Quinn et al., 2016; Yogev et al., 2020). The resulting biogas can be combusted to produce electricity and heat, while the remaining nutrients in the supernatant can be applied as fertilizer (Zhu et al., 2022). The carbon cycle and footprint of the aquaponics systems, especially the carbon dioxide, which is a significant contributor to global warming, have not yet been considered by other studies. This study aimed to investigate the performance of a novel off-grid aquaponics system with zero water and waste discharge, focusing on the carbon cycle and energy recovery that was achieved by the addition of onsite anaerobic treatment of the solid waste streams.

Materials and Methods

The experiments were carried out in a desert aquaponics facility located in a greenhouse with RAS and an adjacent net-house containing deep water culture hydroponics beds at Ben-Gurion University of Negev, Israel. The design of the near-zero waste aquaponics system was based on four treatment loops. Catfish (*Clarias gariepinus*; n=264) was stocked with daily feeding of 2% of the total stocking biomass. Lettuce seedlings (*Lactuca sativa cv. Noga*) were introduced on the floating rafts of hydroponics. Following a stabilization stage, the system was closely monitored for four months. An input-output model to support balances concerning the carbon cycle and footprint in the near-zero waste aquaponics was developed.

Results and discussion

Fish tank water was recirculated via a solid removal unit and nitrification reactor, from which 66% was recycled to the fish tank directly and 34% indirectly through the hydroponically grown plants. Fish stocking density was 15.3 kg/m^3 and over time reached approximately 40 kg/m^3 where it was maintained. Typical fish performance was observed with a survival rate >97% and a feed conversion ratio of 1.33. Results show that 1 kg of feed per day was able to produce about 0.75 kg of fish and support 33.8 kg of fresh lettuce. Fish sludge and plant waste were anaerobically treated, energy was recovered, and the nutrient-rich supernatant was recycled to the plants to enhance production. Lettuce production was up to 5.65 kg/m^2, significantly higher than previous reports, largely because of high nutrients reuse efficiency from the anaerobic supernatant that contained 130 and 34 mg/L N and P, respectively. Of the feed carbon, 24.5% was taken up by fish biomass. Fish solid wastes contained 38.2% carbon, of which 91.9% was recovered as biogas (74.5% CH_4). Biogas production was 0.84 m^3/kg for fish sludge and 0.67 m^3/kg for dry plant material. CO_2 sequestration was 1.4 higher than the feed carbon, which reduced the system's carbon footprint by 64%. The energy consumption per ton of fish stocked in this system is expected to be 84.4 kWh/day, and the potential energy recovery from the waste via AD will be about 83.7 kWh/day, with 85% energy use efficiency of CHP from the biogas to support about 84% of the energy demand for its operation. This study is the first to demonstrate highly efficient fish and plant production with near-zero water and waste discharge and with energy recovery that can potentially supply the system's energy demand.

Conclusions

A near-zero waste desert aquaponics system, which combined aquaponics and anaerobic digestion technologies into a closed system with a small environmental footprint, was successfully demonstrated. The current study is the first to demonstrate a significant recovery of water, energy, and nutrients from aquaponics waste streams. Alternatively, seen from a broader perspective, this concept would allow fish and vegetable production in nontraditional agricultural land, such as rural areas with insufficient electricity supply or in urban environments close to the customer.

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MICROALGAL-CONTAINING DIETS: A SUSTAINABLE AND HEALTH-PROMOTING ALTERNATIVE IN BARRAMUNDI PRODUCTION

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Introduction

Aquaculture is currently the fastest growing animal food production sector, driven by the increased human consumption and market acceptance. Of the major constrains to sustainable production by aquaculture is the incorporation of fish meal and fish oil (FMFO) from capture fisheries, as key ingredients in fish feed in general and in carnivorous fish in particular. FMFO provides essential nutritional components such as amino acids, phospholipids, and fatty acids (such as the omega-3 LC-PUFA docosahexaenoic acid, DHA, and eicosapentaenoic acid, EPA) for optimal development and growth. The FMFO industries are one of the few major animal industries, which still rely on hunting-and-gathering technique. Thus, most fish rendered into meal and oil are captured at sea, which is unsustainable. By this practice millions of tons of FMFO is produced worldwide, posing a significant associated environmental damage. The annual supply of FMFO is presently 6.0 to 6.5 million tons.

Microalgae are primary producers of high-valued biomolecules, such as lipids, proteins, pigments, polysaccharides, LC-PUFA, all with beneficial health-related properties and can therefore serve as a sustainable alternative to FMFO in fish nutrition. n-3 LC-PUFA, EPA and DHA, which are produced by certain microalgae, have immunomodulatory properties and therefore hold major health benefits for humans and fish.

The aim of this study was to investigate the use of microalgae as a substitute for FMFO for feeding barramundi (*Lates calcarifer*), a carnivore fish. Microalgae with specific composition of *n*-3 LC-PUFA were selected to provide the required composition of these fatty acids in the fish feed.

Methods

Microalgae-formulated diets (NI and NIP) containing a combination of *Nanochloropsis oceanica* (N), *Isochrysis galbana* (I) and *Porphyridium purpureum* (P), and no added FMFO were prepared. The diets combining the different microalgae species were constructed to achieve 50% crude protein and c*a*. 11% lipid, by the following composition: NI - containing 7.5% N; 12.5% I; NIP diet - containing 7.5% N; 12.5% I; 3.13% P; and a commercial control diet which contained fish oil. The dietary trial was conducted in a RAS system where juvenile Barramundi (*Lates calcarifer*), with an average weight of 24 ± 0.9 g were stocked in 250 L tanks (1.4 kg/tank). Each dietary group consisted of three replicate tanks (total of nine experimental tanks). Fish were fed with the experimental diets for a period of 8 weeks at 2% of their body weight once a day.

Fish weight was monitored weekly and at the end of the trial fish were sampled and used for the following analyses: 1) whole body proximate composition (moisture, ash, protein, fiber and total lipid contents); 2) blood samples were collected for plasma immunoassays (lysozyme, antiprotease, and myeloperoxidase activities); 3) qRT-PCR based gene expression analysis from head kidneys and blood leukocytes; 4) Histological analysis of internal organs; 5) Microbiome analysis of the gut. In addition, 15 fish from each tank (45 fish per treatment) were challenged with *Vibrio harveyi* by intraperitoneal (IP) injection (100 μl of 10^8 cfu ml^{-1}). Mortality was monitored for 10 days.

Results

Fish fed the microalgae-based diets demonstrated significantly better growth performance (Figure 1a,b). Microalgae-supplemented diets also led to significantly higher survival following bacterial challenge. Immunoassays carried out on fish plasma samples revealed enhanced lysozyme and antiprotease activities in fish fed NI and NIP diets as compared to control. Further analyses of the samples collected are underway.

Our results demonstrate that the mixture of microalgae meal used in this study can improve growth metrics and boost immune defense. We demonstrate that the LC-PUFA rich algal meal can be used in diets of barramundi as an alternative ingredient to fish oil. Our research efforts is a step towards eliminating dependence on fish meal oil, and to improve sustainability of the farmed fish.

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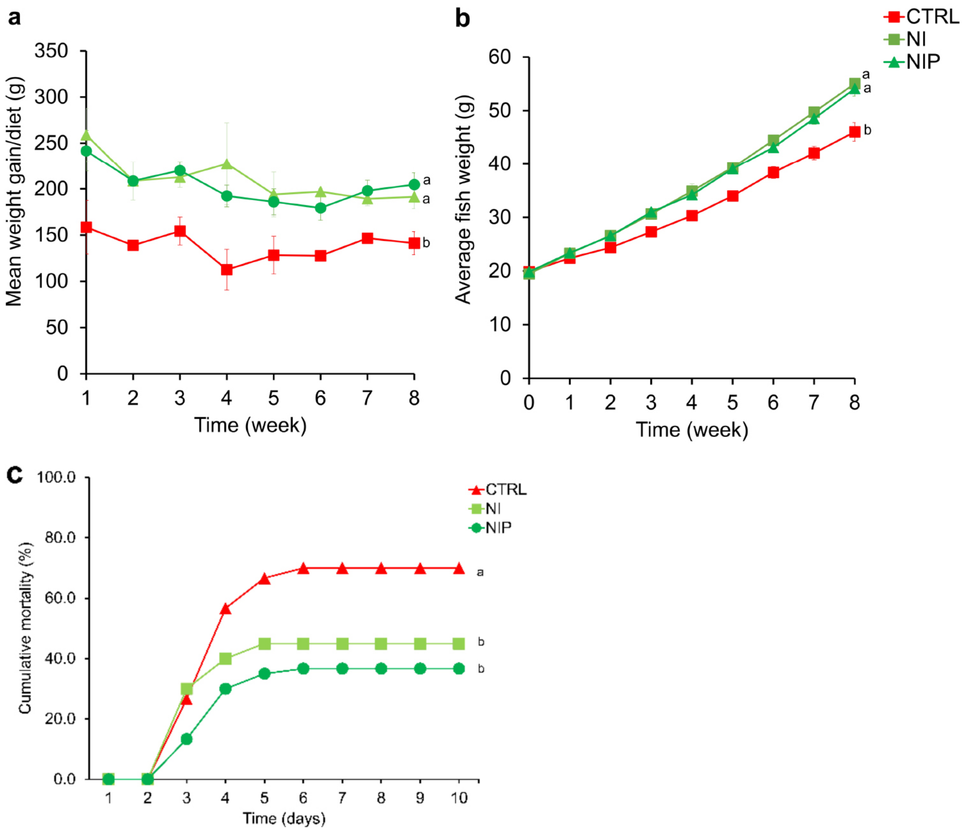

Figure 1. Fish growth performance and resistance to experimental bacterial challenge during the dietary trial period. **a)** weekly weight gain **b)** average fish weight. **c)** cumulative mortality following bacterial challenge. (n=3 tanks). Different letters (a, b) indicate statistical significance between groups ($p<0.05$;)

Acknowledgment: We would like to acknowledge Amity Tal, from Zemach Regional Industries, Zemah, Israel, for his professional input in planning the experimental diets and his expert advice in nutritional-related issues.

FROM FISH EXCRETIONS TO HIGH-PROTEIN DIETARY INGREDIENT: FEEDING INTENSIVELY CULTURED BARRAMUNDI (*Lates calcarifer*) A DIET CONTAINING MICROBIAL BIOMASS, ORIGINATING FROM A MICROAEROPHILIC ASSIMILATION REACTOR OF A RECIRCULATING AQUACULTURE SYSTEM (RAS)

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Introduction

Fish production by recirculating aquaculture systems (RAS) is rising. As fish recover only about 20-30% of the applied feed as biomass, a novel reactor which assimilates the excreted nitrogen into microbial biomass was developed (Yogev and Gross, 2019). This novel reactor reduces the environmental impact of water treatment by nitrification and the discharge of nitrogen through effluents, as done in conventional RASs, as well as enables utilization of the high-valued nitrogen by production of high-protein microaerophilic microbial biomass (MMB) as a feed ingredient. Since feed is the most expensive component in aquaculture, this approach holds an economic potential.

Methods

For the production of MMB by the RAS assimilation reactor, an external carbon source is required to achieve a minimal C:N ration of 15, for which wheat, sugar (SUG) and semolina (SEM) were used. MMB was harvested daily, oven dried (50 ºC), powdered (Fig 1) and used as a feed ingredient for barramundi (*Lates calcarifer*) in two separate trials:

Trial 1: experimental diet was prepared by replacing 20% (w/w) of commercial barramundi feed with dry MMB (wheat as a carbon source) and commercial feed as control.

Trial 2: four reconstructed diets containing different biofloc levels of 15 and 25 % from semolina and sugar as carbon sources were used (15% SEM, 25% SEM, 25% SUG and un-supplemented control).

The trials were conducted in 100 L plastic tanks stocked with barramundi (average weight 27± 0.9 g) at a density of 20 kg/m^3, in three replicates. Feed was applied at 2% body-weight per day. Fish were fed for a period of 8-10 weeks, during which their growth was monitored.

At the end of the trial, the whole-body proximate composition, serum immune function, gene expression were analysed. Fifteen fish from each treatment were challenged with *Vibrio harveyi* to examine their disease resistance.

Results

Survival was 100% in all treatment groups. In both studies, weight gain was significantly ($p < 0.05$) higher in control-fed barramundi as compared to biofloc-fed groups and negatively correlated with the inclusion levels. Feed conversion ratio (FCR) in all treatment groups ranged between 1 to about 2. It did not significantly differ between the treatment and control groups in trial 1 (1.36 and 1.09, respectively; $p>0.05$) but in the 25% biofloc diet in trial 2, FCR was significantly elevated as compared to 15% biofloc and control food (2.03, 1.41 and 1.19, respectively; $p<0.05$). Fish composition was not affected by the different diets.

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Figure 1: Biofloc preparation for incorporation in the feed (left to right): Wet biofloc after centrifugation (supernatant discarded); Oven-dried biofloc (50 ºC); Ground biofloc.

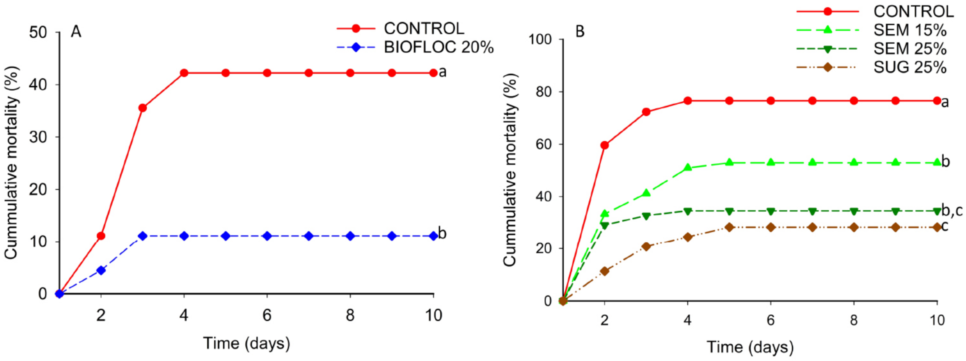

Figure 2: Mortality of fish challenged with V*ibrio harveyi* in trials 1 (A) and 2 (B). Data presents daily cumulative mortality of fish fed with control and treatment diets (n=3 replicate tanks, 15 fish per tank).

Biofloc inclusion improved barramundi resistance to bacterial infection, and higher survival was noted in both trials (Fig 2). Analysis of fish sera showed higher activities of immune factors including myeloperoxidase and lysozyme as compared to control-fed fish in both trials. Results of qPCR revealed significantly higher expression of five immune-related genes (galectin, C-type lectin, hepcidin, lysozyme and complement) in the kidneys of the biofloc-fed fish.

Results demonstrate that biofloc can be used as a feed ingredient in carnivorous fish. Selected levels should be considered to optimize production based on growth and survival performances.

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HIGH-THROUGHPUTSCREENINGOFMICROALGAEFORWASTEWATERTREATMENT AND *In Vitro* EVALUATION OF BIOACTIVITY OF SELECTED MICROALGAE STRAINS

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Introduction

Microalgae exhibit tremendous potential to be an environmentally sustainable solution for bioremediation as they have the capacity of efficiently recovering nutrients from agricultural wastewaters, while simultaneously producing high value bioproducts with various biotechnological application[1,2]. *Chlorella vulgaris, Scenedesmus quadricauda* and *Haematococcus pluvialis* are three widely cultivated freshwater microalgae strains with established commercial exploitation due to their high added-value biomass which we have shown to be effectively cultivated in hydroponic effluents.

Material and Methods

Three widely cultivated strains, *Chlorella vulgaris 211/11B* (CCAP)*, Scenedesmus quadricauda 276/21* (CCAP) and *Haematococcus pluvialis 34/7* (CCAP) were grown under steady conditions (temperature: 24±1˚C, photoperiod: 12:12h L:D, light intensity: 150 μmol photon $m^{-2}s^{-1}$) for 5 days and inoculum re-cultivated in three different culture media: L1 (control medium), PR (hydroponic effluent) and PR-L1 (hydroponic effluent enriched with the micronutrients mixture used to create the nutrient medium L1). Moreover, two aqueous (CVW and SQW) and two ethanolic (CVE and SQE) extracts were prepared by freeze-dried biomass of *C. vulgaris* and *S. quadricauda,* respectively. Total biochemical characterization was determined to all extracts. *In vitro* protective role of the extracts against H_2O_2 induced Caco2 cells was estimated by MTT assay. Subsequently, total RNA was isolated from Caco2 cells exposed to H_2O_2 after a pre-incubation with CVE (0.5μg/ml) and SQW (1μg/ml) extracts. Antioxidant defense pathway and inflammation related genes were selected for analysis.

Results

Our results revealed that all microalgae species included in this study were able to efficiently grow in hydroponic water effluents utilizing the available nutrients (Fig.1)

The biochemical characterization of custom produced extracts from the respective biomass (Table 1) highlighted its potential to be used as bioactive supplement in many applications. To this end, the ability of these extracts to protect Caco2 cells against H_2O_2 induced oxidative stress was demonstrated (Fig.2)

Moreover, the observed protective effects could be clearly attributed to the modulation of the transcription of several human genes involved in oxidative stress responses, by both the CVE and SQW microalgae extracts (**Table 2**).

Conclusions

All the strains when cultivated using the PR and PR-L1 media exhibited similar and sometimes even higher growth rates. CVE and SQE extracts present greater antioxidant activity, flavonoid and pigment content. CVE (0,5 μg/ml) present no cytotoxicity on Caco2 cells and upon 24 hours incubation enhanced viability of H_2O_2-treated cells to 103% whereas SQW (1μg/ml) enhanced viability of H_20_2 treated cells up to 92%. Transcriptome analysis of Caco2 cells pre-treated with CVE and SQW followed by H_2O_2 induced oxidative stress revealed significant alternation at oxidative stress and inflammation related genes expression.

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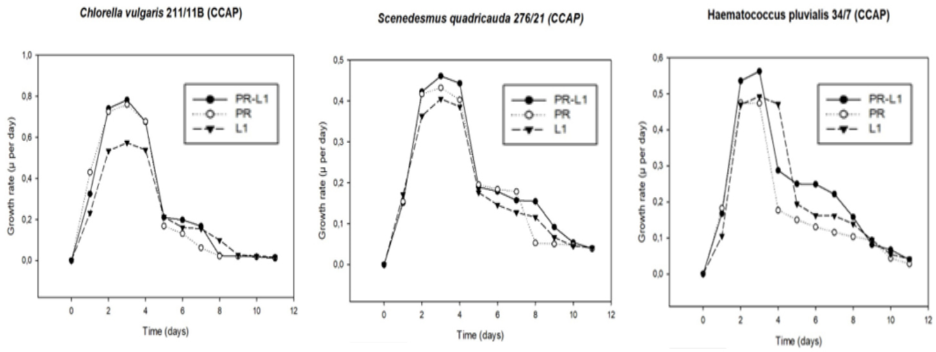


Fig.1 Growth rate of microalgae strains in 3 different culture media (L1, PR, PR-L1)

	CVE	CVW	SQE	SQW
FRAP (μg/ml)[a]	16,27 ± 0,343	2,26 ± 0,009	13,69 ± 0,187	0,71 ± 0,001
TEAC (μg/ml)[b]	19,25 ± 0,380	16,73± 0,086	15,93 ± 0,248	12,66 ±0,058
DPPH (μg/ml)[b]	6,32 ± 0,063	5,96 ± 0,113	3,31 ± 0,145	1,15 ± 0,315
Total phenolics (μg/ml)[c]	11,08 ± 0,144	12,02 ±0,526	6,23 ± 0,058	4,97 ± 0,052
Total flavonoids (μg/ml)[d]	8,53 ± 0,250	3,27 ± 0,232	6,09 ± 0,400	0,40 ± 0,020
Total carotenoids	0,51 ± 0,026	0,04 ± 0,003	0,06 ± 0,004	nd
Chlorophyll A	0,66 ± 0,020	0,27 ± 0,006	0,16 ± 0,0205	nd
Chlorophyll B	0,44 ± 0,024	0,19 ± 0,014	0,11 ± 0,025	nd

Table 1. Values are given as mean ± SE (n=3), [a]As ascorbic acid equivalent, [b]As trolox equivalent, [c]As gallic acid equivalent; [d]As quercetine equivalent.

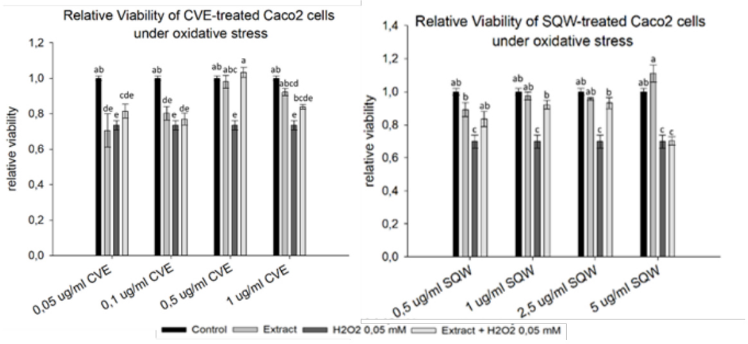
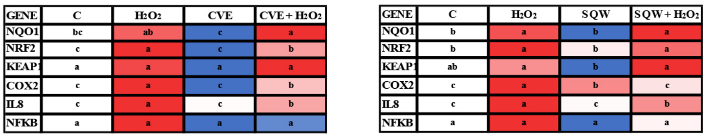


Fig. 2 MTT assay on Caco2 cells after 24h incubation with CVE and SQW under 0.05 mM H_2O_2 (3h incubation). Letters indicate significant differences according Tukey's range test ($p < 0.05$).



GENE	C	H_2O_2	CVE	CVE + H_2O_2
NQO1	bc	ab	c	a
NRF2	c	a	c	b
KEAP1	a	a	a	a
COX2	c	a	c	b
IL8	c	a	c	b
NFKB	a	a	a	a

GENE	C	H_2O_2	SQW	SQW + H_2O_2
NQO1	b	a	b	a
NRF2	b	a	b	a
KEAP1	ab	a	b	a
COX2	c	a	b	c
IL8	c	a	c	b
NFKB	a	a	a	a

Table 2 Relative expression of oxidative stress and inflammation related genes. Caco2 cells were pretreated with CVE and SQW extracts for 24 hours. Oxidative stress was induced with 3 h incubation with H_2O_2 0.05 mM. Letters indicate significant differences according Tukey's range test ($p < 0.05$). Red color represents the upregulated genes; the higher intensity of color denotes greater fold-change; blue represents the downregulated genes.

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ENVIRONMENTAL IMPACT OF OFF-SHORE AQUACULTURE: LIFE CYCLE ASSESSMENT OF SEABASS AND SEABREAM PRODUCTION

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Introduction

Globally, aquaculture is the fastest growing agri-food sector and it is increasingly seen as the primary activity to meet the growing consumer demand for fish. Up to now, European seabass (sp. *Dicentrarchus labrax*) and Gilthead seabream (sp. *Sparus aurata*) are the most commonly farmed species in the Mediterranean Sea, with a production of 464,000 tons and USD 2.24 billion in 2019 (FAO, 2020). However, intensive fish farming, implies environmental concerns related to the consumption of feed, the emission of nutrients and organic compounds into the water and, sometimes, the consumption of pesticides and antibiotics for pest and disease control (le Feon et al., 2021). Developing sustainable aquaculture is a priority in the current economic and social context. Therefore, aquaculture production systems must change to improve environmental performance and decrease energy consumption. To achieve these objectives, a holistic perspective is needed considering the impacts generated by production, and Life Cycle Assessment (LCA) approach is considered the most suitable tool for analyzing such a wide spectrum. In this study, LCA was applied to assess the environmental impact related to Seabass and Seabream farming of an off-shore plant located in Central Italy.

Materials and method

The selected functional unit (i.e., the reference unit of the study to which all the inputs and outputs should be referred) was 1 ton of Seabass and 1 ton of Seabream at the fish farm gate. Moreover, the different feeds supplied were analysed and the impact of 1 ton of different feeds calculated. The system boundary includes the production of feed and other production factors consumed (e.g., fuel, electricity), the rearing operations and all the emissions related to the process (e.g., phosphorous and nitrogen emissions due to the metabolism of the fish during the entire production cycle). The inventory data was collected in a commercial aquaculture plant in Central Italy. Primary data refers to the consumption of different feeds based on the sizes of fish, energy and fuels while secondary data was used with regard to the emissions of N and P compounds from fish (Cho et al., 1991), fry production (Garcia Garcia et al., 2019) and feed composition (estimated with a centesimal analysis based on the content of proteins and fats). Background data about cages and other capital goods as well as about the component of the feed were retrieved from databases (Ecoinvent® and Agrifootprint). The environmental profile of the production system was analysed with the Recipe Midpoint (H) method, taking into account 12 different impact categories.

Results

For European Seabass and Gilthead Seabream, the environmental results, for most of the considered impact categories, showed that aquafeed is the main environmental hotspots. The species analysed show the same results as regards the analysis of contributions. For the Climate Change impact category, aquafeed impacts about 60% of the total impact, while for marine eutrophication the emissions of N compounds accounts for 95% of impact. Infrastructures have an high impact in the human carcinogenic toxicity (75%) and mineral resource scarcity (59%). Comparing the two species, Seabass has higher impacts in all impact categories than Seabream: this is mainly due to the higher FCR of Seabass than Seabream (respectively 2.4 and 1.9), which also results in higher emissions of nitrogen compounds per kg of fish produced. In addition, Seabass has a higher average mortality rate (20% vs 8% for Seabream): therefore, a greater number of fry is necessary to obtain the same production. Finally, the feeds that contain a greater quantity of fish meal and fish oil, supplied to smaller sized fish, have the highest impacts.

Conclusions

This study confirmed that aquafeeds management is the main responsible for most of the impacts. Measures that optimize the production and use of fish feed are necessary and can positively affect the environmental performances of aquaculture sectors. Similar results, both in absolute terms and in terms of relative contributions, are reported by other studies (Aubin et al., 2009; Abdou et al., 2017). To date, the large amount of protein and lipids required for fish growth is mainly provided by fishmeal and fish oil from freshly caught fish stocks. Therefore, it is essential to optimize and modify diet formulation

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as well as to study alternative feeding strategies in order to lower FCR. In addition, aquaculture requires a very high consumption of fossil fuels (both for the mobility of ships and for the supply of feed and fry); for this reason, following the example of the SIMTAP system developed during the project SIMTAP (Self-sufficient Integrated Multi-Trophic AquaPonic systems for improving food production sustainability and brackish water use and recycling), new diets characterized by limited transportation impacts and by the use of locally produced raw materials, combined with the maximization of the use of renewable energy (e.g. solar energy) and alternative protein sources (e.g. microalgae) can represent other effective impact mitigation strategies. Finally, besides the environmental performances also the economic and social ones should be evaluated for a more comprehensive assessment of the process sustainability

Acknowledgements

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CARRYING CAPACITY ESTIMATION IN GREEK COASTAL CAGE AQUACULTURE

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Introduction

The estimation of the carrying capacity (CC) is a fundamental process in integrated environmental management, policy making, and decision making. Aquaculture carrying capacity has been studied since the 1960s to allow estimation of the production limits of aquaculture projects and, hence, their maximum economic performance within sustainable limits for the local environment. One major drawback of these approaches is that they can provide CC estimates after a fish farm is installed and operates in a certain location (ex post approaches). This work approaches the estimation of CC using a Bayesian/CHAID model of profiling information on the environmental quality, geomorphology, and human activities on the adjacent coastal area (land side) using as an indicator the trophic state of the marine area in terms of chlorophyll-*a* concentration (upper meso- trophic).

Material and methods

Eight marine cage farm sites in Greece (Sterea Ellada region; Figure 1) were selected, for the collection of a variety of physico-chemical data: temperature, salinity, dissolved oxygen (DO), dissolved inorganic nitrogen (DIN = ammonium - NH_4^+ +nitrates - NO_3^- + nitrites - NO_2^-, soluble reactive phosphorus (PO_4^{-3}), total nitrogen (TN), total phosphorus (TP), chlorophyll-a (Chl-a), suspended particulate matter (SPM), total organic carbon (TOC), particulate organic carbon (POC), particulate organic nitrogen (PON) and particulate organic phosphorus (POP). Water samples were taken at 1 m below the surface and 1 m above the bottom, on a monthly basis, during the period March 2015 - May 2016. Moreover, geomorphological data (depth, watershed, fetch, openness, coastal area, exposure and water retention time), a farm operational parameter (biomass), and a social parameter (local inhabitants) were also used.

Results

The application of the Bayesian–CHAID algorithm, based on the monthly data of all parameters measured, showed that the CC of the studied fish farm areas (measured as the maximum cultivated biomass so that the eutrophication level remains always at the upper mesotrophic level or below), is affected by the following parameters (ranked from most important to least important): particulate nitrogen, salinity, silicates and suspended particulate matter and the overall relationship found for the biomass carrying capacity is:

$$\text{Biomass at CC level} = +473.762[\text{Chl-a}] - 6856.64[\text{PN}] + 9.302[\text{Salinity}]$$
$$- 473.5[\text{Si-SiO4}] + 341.864[\text{SPM}] - 207.046$$

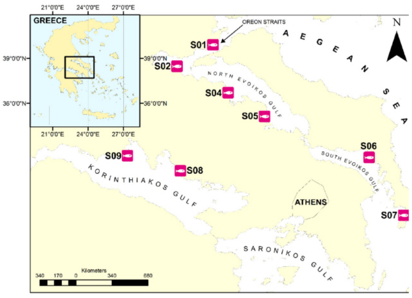

Figure 1. Farm sites location

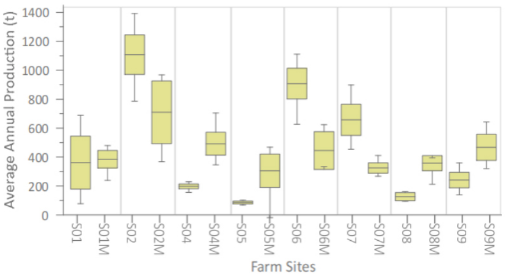

Figure 2. Annual average actual production and the model predictions for each farm site (letter M in y-axis labels denotes the model estimates).

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The application of the model to the current farm sites shows that from the 8 farms studied, only S01 should maintain their level of annual production to achieve the environmental goal of a eutrophication level up to 3 (upper mesotrophic). Of the rest farms S02, S06, and S07 should reduce their average production, while farms S04, S05, S08, and S09 can easily increase their average production and almost double it (Figure 2).

In addition, the analysis performed allowed us to estimate the maximum levels for each factor to maintain a eutrophication status up to the upper mesotrophic level which are:

particulate nitrogen < 0.018 mg/L, silicates < 0.137 mg/L, salinity > 38 psu and
SPM > 0.815 mg/L

Conclusions

The use of the Bayesian–CHAID model for the estimation of the maximum CC of a given coastal area for cage fish farming includes factors related to natural pressures on the coastal area. Most of the PON originates in inland waters as a result of the human production of food and energy and occur in the plankton [2], salinity is affected by the section area and governs the plankton species survival [3], silicates concentration depends on river out flows and affects the diatoms concentration [4] and SPM is related with the adjacent land and land-originating effluents and is consider a potent driver for plankton growth [5].

The analysis performed allowed us to estimate the maximum levels for each factor to maintain a eutrophication status up to the upper mesotrophic level

A significant utility from the specific model application is the creation of a formula which can be used by anyone with different levels of education or expertise (for example, public administrators).

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THE EFFECT OF WATER DISINFECTANT PREPARATIONS ON THE HEALTH CONDITION OF CARP (*Cyprinus carpio* L.)

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Introduction

Water quality is a limiting factor in fisheries management and significantly impacts fish health and production level. When water quality has deteriorated, it is necessary to focus on its treatment and thus prevent potential losses caused by the introduction of pathogenic agents into the breeding environment. In recent years, it has become more and more challenging to have sufficient, safe water available to fill large-volume storage pond systems. Sophisticated treatment of pathogen-laden water from adjacent stocked locations appears to be an economic element in the entire rearing process. Chlorine-containing preparations (Chloramine T and Calcium hypochlorite) and preparations based on peracetic acid appear to be suitable disinfectants for water treatment and the elimination of pathogens. All three disinfectants do not leave residues in the environment. Although they have a low therapeutic index, which indicates the ratio of the concentration of the preparation causing death in fish (LC50) to the dose causing a therapeutic effect (EC50), their residual amounts in water can be used for preventive treatment of fish. The goal of the study was to verify the effectiveness and efficiency of water disinfection treatment in order to reduce the infectivity of the burdened environment, and the morbidity of the fish kept. The health status of the fish was evaluated on the basis of the hematological and biochemical parameters of the fish, as well as the parasitic and bacterial examination of the fish after exposure to disinfectants.

Material and Methods

A total of 60 fish with an average weight of 25 g was divided into 12 aquariums (5 fish per aquarium) with a water volume of 20l. Fish were exposed to the effects of selected disinfectants for three days, as follows:

- Control group – 3 aquariums - fish left in clean tap water
- CHT group – 3 aquariums - fish exposed to chloramine T at a dose of 20 mg/l
- KPO group – 3 aquariums - fish exposed to 1 mg/l peracetic acid
- CH group – 3 aquariums - fish exposed to 1.5 mg/l of calcium hypochlorite

The application of Chloramine T and calcium hypochlorite to the water was made immediately before placing the fish in the aquarium. The KPO application was carried out once a day, three times in total.

After three days of exposure, blood was taken from the caudal vessel of the fish, then they were killed, and a bacteriological smear of the skin and a parasitological examination were performed. The obtained results were statistically evaluated.

Results

By evaluating the hematological parameters, only an increased hematocrit was recorded in the CHT group compared to the control. The most statistically significant changes in biochemical indicators were also recorded in the CHT group, where Glu, ALP and PHOS were different. The parasite examination revealed a positive effect of all used disinfectants on the reduction or elimination of the parasites found, with the exception of the parasite *I. multifiliis*. The CH group showed the lowest efficiency against parasites. Semiquantitative bacterial examination showed a statistically significant reduction of bacteria in the CH and KPO groups compared to the control.

Conclusion

Considering the analysis results, KPO appears to be the universal disinfectant, having an effect on both parasitic and bacterial colonization with a minimum of observed side effects on the fish organism.

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